# FINAL REPORT

## Monitoring Species of Concern Using Noninvasive Genetic Sampling and Capture-Recapture Methods

## ESTCP Project RC-201205

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Dr. Lisette Waits
Dr. Robert Lonsinger
Dr. Susannah Woodruff
**University of Idaho**



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Through this project we demonstrated the effectiveness of an approach utilizing noninvasive genetic sampling combined with capture-recapture methods (NGS-CR) to evaluate the status of kit fox (*Vulpes macrotis*) populations on Dugway Proving Ground (DPG) and Sonoran pronghorn (*Antilocapra americana sonoriensis*) on the Barry M. Goldwater Range (BMGR). We compared the reliability of demographic parameters and the cost-efficiency of NGS-CR approaches with alternative approaches. We developed a spatio-temporal sampling design for acquiring noninvasive genetic data (via fecal scats) from individuals, genotyped samples for individual identification, analyzed genotypes with capture-recapture methods to obtain estimates of key population parameters, and developed a protocol for long-term monitoring in the future. We also quantified expenditures to examine cost efficiency of the approach. For both species, we concluded the NGS-CR method provided reliable estimates, improved monitoring (i.e., increased the number of demographic parameters estimates), and improved monitoring efficiency. Survey results indicated managers and technicians NGS-CR methods were easier to implement than traditional methods (e.g., radio telemetry). Managers at these installations have expressed interest in continuing using these NGS-CR methods.

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| a. NAME *(Last, First, Middle Initial)* | b. OFFICE/AGENCY | c. TELEPHONE NO. *(Include Area Code)* |
|---|---|---|
| Leeson, Andrea | Deputy Director and ER Program Manager | 571-372-6398 |

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WHEN CLEARED, PLEASE EMAIL DD-1910 to QuYahni.D.Lewis.ctr@mail.mil.

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## ACRONYMS AND ABBREVIATIONS

ADO – Allelic Dropout

AICc – Akaike's Information Criterion with small sample size correction

AZGFD – Arizona Game and Fish Department

BMGR – Barry M. Goldwater Range

*c* – Probability of Recapture

CPNWR – Cabeza Prieta National Wildlife Refuge

*D* – Estimated Density

DoD – Department of Defense

DNA – Deoxyribonucleic Acid

DPG – Dugway Proving Ground

ECM – Equal Capture Model

ε – Probability of local extinction

ESA – Endangered Species Act

FA – False Allele

γ – Probability of colonization

GIS – Geographic Information Systems

*g0* – Detection Function Intercept

GPS – Global Positioning System

ID – Identification

INRMPs – Integrated Natural Resource Management Plans

KNN – *k*-Nearest Neighbors

LAFB – Luke Air Force Base

MNKA – Minimum Number Known Alive

mtDNA – Mitochondrial DNA

nDNA – Nuclear DNA

$N_e$ – Effective Population Size

NGS – Noninvasive Genetic Sampling

NGS-CR – Noninvasive Genetic Sampling – Capture Recapture

NGS-OM – Noninvasive Genetic Sampling – Occupancy Modeling

NWR – National Wildlife Refuge

*p* – Probability of Capture

P(ID)sibs – Probability That Two Siblings Have Identical Multilocus Genotypes

PCR – Polymerase chain reaction

$\psi$ – Probability of occurrence (occupancy)

$\sigma$ – Detection Function Scale Parameter

$S$ – Probability of Survival

$S_F$ – Female Probability of Survival

$S_M$ – Male Probability of Survival

SECR – Spatially Explicit Capture-Recapture

$\sigma$ – Detection Function Scale Parameter

TIRM – Two-Innate Rates Model of Capture

SERDP – Strategic Environmental Research and Development Program

USFWS – United States Fish and Wildlife Service

USU – Utah State University

YPG – Yuma Proving Ground

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## EXECUTIVE SUMMARY

### OBJECTIVES OF THE DEMONSTRATION

Department of Defense (DoD) lands support the greatest densities of species of conservation concern among lands managed by federal land management agencies. The DoD is required to protect and provide for the conservation of these species, while adhering to the military mission.

To facilitate these efforts and evaluate the effects of management or training actions, the DoD needs reliable, accurate and cost-effective methods for assessing and monitoring species of concern while minimizing impacts on military training. Specifically, managers and biologists need methods for estimating population distribution, abundance, survival, reproduction, movements, and genetic diversity. Our primary objective was to demonstrate how noninvasive genetic sampling (NGS) could be efficiently combined with capture-recapture modeling (NGS-CR) to evaluate the status of species of conservation concern. The main advantage of NGS-CR is that species can be inventoried and monitored by collecting DNA from the natural environment (e.g., hair, feces, feathers) without the need to disturb, capture, or even see the animals (Taberlet et al. 1999, Beja-Pereira et al. 2009), an important consideration when working with endangered, threatened, or imperiled species. Additionally, many recent studies have opted for NGS-CR due to logistical considerations or improved cost-benefit (e.g., Prugh et al. 2005, Boulanger et al. 2008, Kendall et al. 2008, Meijer et al. 2008, Marucco et al. 2009, Broseth et al. 2010, Stenglein et al. 2010b). A secondary objective was to demonstrate how NGS could be combined with occupancy modeling (NGS-OM) to estimate the proportion of area occupied (i.e., occupancy) and patterns of local extinction and colonization for species of conservation concern. One benefit of NGS-OM is that it requires only species identification of noninvasively collected genetic samples, as opposed to NGS-CR, which requires individual identification. Consequently, NGS-OM may offer a more affordable monitoring strategy for species of conservation concern if estimates of abundance and survival are not required.

Despite the widely recognized need for reliable monitoring data, many species of vertebrates are difficult to monitor because they are wide-ranging, occur in low densities, or are otherwise not amenable to traditional methods. For example, some of the more common approaches (e.g., telemetry, capture-recapture, aerial surveys, point counts) for monitoring bird and mammal populations rely on direct visual observation and/or physical capture of animals, are logistically difficult, and can be expensive and time consuming to implement at large spatial scales over long time frames. Other methods (e.g., scent-station surveys, spotlight counts, scat deposition rate surveys, and activity indices) may be more practical to implement but provide only indices of abundance, neglecting important information on actual population size, survival, reproduction and movements (Long et al. 2008). Thus, there is great potential for the DoD to benefit (i.e., relaxed restrictions on training and land use, increased cost efficiency, and enhanced species conservation) from implementing the most effective and innovative approaches for monitoring populations.

We evaluated the efficacy of NGS-CR as a viable, long-term monitoring approach for two species on DoD installations: the kit fox (*Vulpes macrotis*), a species of concern for western installations, and Sonoran pronghorn (*Antilocapra americana sonoriensis*), an endangered subspecies of North American pronghorn that occurs in southern Arizona. Demonstrations for kit foxes and Sonoran pronghorn were conducted at Dugway Proving Ground (DPG) and Barry M. Goldwater Range (BMGR), respectively. These species were selected because they (1) represented species with disparate sampling requirements (dispersed transect sampling for kit foxes vs. sampling at aggregation points [i.e., at drinkers] for Sonoran pronghorn) and (2) were being monitored with more traditional approaches, which provided alternative estimates of population parameters for comparison. We developed a spatio-temporal sampling design for acquiring noninvasive genetic data (via fecal scats) from individuals, genotyped samples for individual identification, analyzed genotypes with capture-recapture methods to obtain estimates

of key population parameters, and developed a protocol for long-term monitoring in the future. We also quantified expenditures to examine cost efficiency of the approach. We evaluated NGS-OM only for kit foxes, and its sympatric intraguild predator, the coyote (*Canis latrans*), at DPG. We implemented a sampling design for acquiring canid scats that allowed us to monitor kit foxes and coyotes simultaneously, used genetic analyses to confirm species, and employed dynamic occupancy modeling to obtain estimates of detection, proportion of area occupied, and dynamic parameters (i.e., local colonization and local extinction) for each species, and to evaluate the influence of coyotes and landscape features on kit fox space-use. We compared the cost of monitoring with NGS-OM to monitoring with NGS-CR.

Our performance objectives were to (1) improve monitoring protocols for kit foxes and Sonoran pronghorn based on NGS-CR, (2) obtain reliable estimates of demographic parameters from NGS-CR for each species, (3) improve efficiency of current monitoring programs, (4) evaluate ease of use, (5) obtain estimates of occupancy and dynamic parameters (i.e., local colonization and extinction) from NGS-OM for kit foxes, and (6) facilitate transference of monitoring programs for kit foxes and Sonoran pronghorn based on NGS-CR. Collectively, our results presented in this report indicate all of our performance objectives were met.

## TECHNOLOGY DESCRIPTION

*NGS-CR technology description*

Capture-recapture modeling has been commonly used for estimating population parameters for wild animals. The theory is based on modeling capture and recapture probabilities of populations or individuals as a function of population size, survival, reproduction, and movements among populations. First used to estimate wildlife population abundance by Lincoln (1930), the approach has been advanced to estimate other demographic parameters such as survival, reproduction, and movements. The basic process involves capturing individuals and marking them, such that on subsequent capture occasions, marked individuals can be identified. Using the observed capture histories, demographic parameters can be estimated.

NGS-CR is an attractive approach because noninvasive genetic material provides DNA of free-ranging animals that can be used to identify unique individuals within the population with little or no disturbance to the animals (Solberg et al. 2006, Marucco et al. 2009, DeBarba et al. 2010, Stenglein et al. 2010a). Recorded “captures” of unique genotypes can be used in capture-recapture models to estimate demographic parameters, such as abundance, survival, reproduction, immigration, or emigration.

In this demonstration, we employed Pollock’s robust design (Pollock 1982, Pollock et al. 1990, Kendall et al. 1997) capture-recapture models, which is a powerful sampling design consisting of two types of sampling periods. There are long time intervals (e.g., several months or years) called primary periods when it is assumed that individuals may reproduce, die, or move in or out of the study area. Secondary periods within each primary period are separated by relatively short time intervals (e.g., days or weeks). During this time, the population is assumed to be demographically and geographically closed (i.e., little or no reproduction, mortality, or

movement among subpopulations). The robust design allows for estimation of population size at each primary period, survival and temporary emigration between primary periods, and capture probability during secondary periods.

Additionally, we employed single session 'capture with replacement' (CAPWIRE) models which have been developed specifically for NGS. CAPWIRE exploits repeat detections of individuals within a single sampling occasion to generate abundance estimates (Miller et al. 2005). For kit fox, we also employed spatially explicit capture-recapture (SECR) models. SECR models use spatially disparate captures of individuals to address capture heterogeneity among individuals associated with proximity to the trapping array, and to estimate density by directly delineating the effective sampling area (Borchers and Efford 2008). Data collected via NGS differs from conventional capture-recapture data in that individuals can be captured >1 time within a sampling occasion (Miller et al. 2005, Thompson et al. 2012). SECR models can generate estimates based on a single sampling occasion (when individuals are captured >1 time across spatially disparate locations; Efford 2011).

*NGS-OM technology description*

Patterns of species occurrence are often derived from presence-absence data. Noninvasive sampling strategies may offer increased detection rates for rare or elusive species, reducing costs and minimizing impacts on target species (e.g., Long et al. 2011). Occupancy modeling utilizes information from repeat surveys to account for imperfect detection and produces unbiased estimates of occupancy (MacKenzie et al. 2002, 2003, 2006). Unlike NGS-CR, the unit of analysis in occupancy studies is the survey site (or patch) not the individual. Consequently, patterns of occurrence can be modeled as a function of patch characteristics, such as habitat or landscape features. Because occupancy modeling focuses on the species, analyses do not require individual identification, reducing costs associated with genetic analyses compared to NGS-CR.

Replication is required to estimate probability of detection for occupancy models and may be accomplished through temporal or spatial replicates within a site. Single-season occupancy modeling has become common, but only provides estimates of detection and proportion of area occupied. Alternatively, dynamic occupancy models (i.e., multi-season occupancy models) allow practitioners to assess patterns of colonization and local extinction, which drive the observed occupancy states (MacKenzie et al. 2003, 2006). Like Pollock's robust design, dynamic occupancy models consist of both primary periods and secondary periods. Sampling sites are assumed to be closed to changes in occupancy state within primary sampling periods, while changes may occur between primary sampling periods.

## DEMONSTRATION RESULTS

*Kit fox*

Pilot studies evaluated kit fox and coyote sample (i.e., scat) accumulation and fecal DNA degradation rates (Lonsinger et al. 2015a). The results of these pilot studies were combined to develop a conceptual model for identifying the most efficient (i.e., minimal cost per successful

sample) temporal sampling design for NGS-CR. Degradation results indicated that mitochondrial (mtDNA) and nuclear (nDNA) DNA amplification success rates for kit fox and coyote fecal DNA samples were relatively high. Across species and seasons, mtDNA amplification success was ≥95% through day 21. Kit fox nDNA amplification success was ≥70% through day 21 across seasons. Coyote nDNA success was ≥70% through day 21 in winter, but declined to <50% by day 7 in summer. Mean scat accumulation rates were nearly three times greater for coyotes (0.076 scats/km/day) than kit foxes (0.029 scats/km/day) across seasons. We identified a common temporal sampling frame of ~14 days that allowed species to be monitored simultaneously, further reducing time, survey effort, and costs. Our results suggest that when conducting repeated surveys for NGS-CR analyses, overall cost-efficiency may be improved with a temporal design that balances field and laboratory costs (Lonsinger et al. 2015a).

To inform future monitoring efforts, we evaluated the success of field-based species identification for scats of kit foxes and coyotes, and compared this to the predictive power of two nonparametric classification techniques—*k*-nearest neighbors and classification trees—based on scat measurements (Lonsinger et al. 2015b). Overall, 12.2% of scats were misclassified by field identification, but misclassifications were not equitable between species. Only 7.1% of the scats identified as coyote with field identification were misclassified, compared with 22.9% of scats identified as kit fox. Results from both *k*-nearest neighbor and classification-tree analyses suggest that morphometric measurements provided an objective alternative to field identification that improved classification of the rarer species. Overall misclassification rates for *k*-nearest neighbor and classification-tree analyses were 11.7% and 7.5%, respectively. Using classification trees, misclassification was reduced for kit foxes (8.5%) and remained similar for coyotes (7.2%), relative to field identification (Lonsinger et al. 2015b).

We used experimental plots to evaluate variation in kit fox and coyote scat removal along roads at DPG, which provides valuable information to inform future sampling efforts (Lonsinger et al. 2016. Kit fox scats disappeared more rapidly than coyote scats, with 3.3% and 10.6%, respectively, persisting through 42 days. At 14 days, 90.8–41.7% of scats had been removed across road types. Parametric survival regression models indicated species, road type, scat position and daily traffic were important predictors of scat persistence. Applying persistence-rate correction factors to scat survey results altered the inferred relative abundances. Randomization tests suggested that when removal rates were high, corrected relative abundance estimates could vary substantially (Lonsinger et al. 2016). By evaluating scat removal rates experimentally, as we have done here, practitioners can elucidate those conditions expected to result in exceptionally high removal, and can use this information to identify appropriate survey routes and/or avoid transects with high removal rates.

Kit fox individual identification success rates (i.e., the proportion of samples identified to species for which a successful individual identification was achieved) ranged from 59.4% (summer 2013) to 91.4% (winter 2013). Across sessions, 109 kit foxes were identified, among which 102 individuals had consensus genotypes at ≥8 loci. Sex was determined for all individuals. We captured 36–50 kit foxes each session and 37 individuals across >1 sessions. We captured more males (60%) than females. Male kit fox survival ($S_M$) was slightly lower than female survival ($S_F$) across intervals and overall. Model-averaged kit fox survival was high in the period between winter 2013 and summer 2013 ($S_M$ = 0.82, 95% CI = 0.26–0.98; $S_F$ = 0.87, 95% CI = 0.28–0.99), high between summer 2013 and winter 2014 ($S_M$ = 0.81, 95% CI = 0.19–0.98; $S_F$ = 0.87, 95% CI

= 0.24–0.99), and lower in the interval from winter 2014 to summer 2014 ($S_M$ = 0.59, 95% CI = 0.11–0.94; $S_F$ = 0.67, 95% CI = 0.16–0.96).

We compared likelihood-based estimates of kit fox abundance resulting from (i) robust design non-spatial capture-recapture models, (ii) multi-session spatially explicit capture-recapture (SECR) models, and (iii) single-occasion capture with replacement (CAPWIRE) models (Lonsinger 2015). Estimates of kit fox density from SECR models were similar across sessions (0.018–0.022 animals/km$^2$); these estimates were among the lowest reported in the literature and at DPG. Derived estimates of kit fox abundance from SECR models were generally higher than those from robust design non-spatial models. Still, confidence intervals for the SECR and robust design non-spatial models had considerable overlap, suggesting that both models produced similar estimates. The model-averaged abundance estimates from robust design non-spatial models suggested that there were 60.1–73.2 kit foxes present in the study area and the 95% confidence intervals suggested that population abundance was stable across sessions. Abundance estimates from CAPWIRE were generally lower than those from robust design non-spatial models and SECR models. Single-occasion CAPWIRE models produced estimates of kit fox abundance that were substantially lower (27.5–59.2%) than multi-session estimates, ranging from 30–53.

We employed NGS-OM to evaluate the spatial dynamics of kit foxes and their intraguild predators, coyotes (Lonsinger 2015). Across sessions, naïve estimates of coyote occupancy were >0.7 in all but the first session and probability of occurrence was not significantly different from 1. For kit foxes, naïve estimates of occupancy were ≤0.3, with the probability of occurrence estimated to be ≤0.5. Coyote occupancy was unrelated to water availability, but was positively related to the proportion of shrubland and woodland habitat. Kit fox occupancy displayed an inverse relationship, being negatively related to shrubland and woodland habitat. Kit fox probability of local extinction was positively related to site-level coyote activity, and within an occupied site, the probability of kit fox detection was positively related to transect-level coyote activity (i.e., kit fox detection was higher on transects with more coyote sign). Our results support previous research at DPG that suggested kit foxes distributed themselves to minimize overlap with coyotes at broad scales (Kozlowski et al. 2012), but also suggested that at finer scales, kit foxes may still adhere to expectations of the resource availability hypothesis and may forage in riskier habitats to meet their dietary needs (Lonsinger 2015).

*Pronghorn*

Pronghorn pilot studies included developing a species identification test using mtDNA species-specific primers to distinguish between Sonoran pronghorn and mule deer (*Odocoileus hemionus*) using DNA extracted from fecal pellets (Woodruff et al. 2014). Each species was accurately identified in 100% of the blood and tissue reference samples. In evaluating the rate of DNA degradation in fecal samples ranging from 1 to 124 days old, we documented that mtDNA species identification success rates were 100% through day 14. Success rates dropped to 95% by day 21, 50% on day 60, and 10% by day 124. Average amplification success rates for six nDNA microsatellite loci were 81% for samples on day one, 63% by day seven, 2% by day 14, and 0% by day 60. We also evaluated fecal pellet deposition rates and fecal DNA degradation rates to maximize sampling efficiency for our capture-recapture analyses. Deposition data averaged one pellet pile per pronghorn per day. Based on individual ID success, a sampling interval of 1 to 7

days would be sufficient to optimize amplification success rates; however, an interval of 1 to 3 days would likely give too small a sample size, and local managers attempt to limit pronghorn disturbance at drinkers to once per week. Sampling every 4–5 days is the ideal balance between efficiency of cost, DNA degradation, and deposition. However, we propose using a 7 day sampling interval in order to minimize disturbance and synchronize weekly agency personnel visits for stocking feed and water with fecal DNA sample collection (Woodruff et al. 2015).

To determine the feasibility of distinguishing age class by pellet dimensions, we measured Sonoran pronghorn fecal pellets and matched them to known age animals using fecal DNA genotyping. Based on cross-validation with logistic regression predictive models, we estimated a 98% probability of correct classification of fawn versus yearling and fawn versus adult using pellet width as a single explanatory variable. We could not, however, distinguish between yearlings and adults (Woodruff et al. 2016a).

We estimated abundance for Sonoran pronghorn in 2013 and 2014 and annual survival between 2013 and 2014. Separate population estimates were generated for developed water holes (drinkers) and drinker and non-drinker locations in 2014. The population using drinkers was 116 (95% CI: 102–131) and 121 individuals (95% CI: 112–132) in 2013 and 2014. The population estimate for all locations was 144 individuals (95% CI: 132–157). Adults had higher annual survival probabilities (0.83, 95% CI: 0.69–0.92) than fawns (0.41, 95% CI: 0.21–0.65). Our results provided the first survival estimates for this population in over 2 decades as well as precise estimates of the population using drinkers (Woodruff et al. 2016b).

Lastly, we explored trade-offs between sample size, number of sessions, and multi-session (CMR) versus single-session (*capwire*) closed capture-recapture abundance estimators, and the need for an accurate and precise estimate. In simulations, abundance was biased positively in *capwire* and negatively in CMR. Bias increased with fewer samples/individual/session. Our empirical data had increased precision with more sessions. Our simulation results indicate our empirical estimates are reliable. We recommend collecting 1.5–2 samples/individual/session in ≥2 sessions and the use of a multi-session model, such as CMR. Cost per individual monitored in 2014 was ~$184 USD for NGS-CR methods and $599 USD for aerial sightability methods. However, our results indicate that at the current estimated abundance (~200), the same level of precision (aerial CV ~ 21%) can be obtained using NGS-CR methods for ~$5800, or an annual cost savings of over $4000 (Woodruff et al. In Review).

## IMPLEMENTATION ISSUES

The use of environmental DNA (eDNA) has shown considerable promise for field application in monitoring programs directed at rare and imperiled species. To fully understand the potential for application and limitations of this technology, three main factors need to be considered: 1) production, 2) degradation, and 3) the transport and removal of eDNA. We quantified these factors using pilot studies during the sampling design phase, and we strongly recommend this for other new projects using these methods. We detected differences in deposition rates (production) by season for both species. For Sonoran pronghorn, deposition rates were related to drought intensity, since a greater number of pronghorn used the drinkers as the drought season

progressed. This could lead to challenges in implementation during short, low intensity drought seasons. Also, for Sonoran pronghorn, we were limited to estimating the size of the population using drinkers, which may have differed annually due to inconsistent use of drinkers due to climatic and range (i.e., availability of natural forage) conditions rather than true changes in population size. We do not know with certainty the proportion of the pronghorn population that uses drinkers. However, this would be a very valuable metric and could be estimated by managers through comprehensive monitoring of the proportion of radio-collared individuals using drinkers. We also experienced sampling challenges related to land access (i.e., ability to access all sampling areas in a timely manner) for both species. Patterns of animal and vehicular activity can also influence the rate of removal by destroying samples. We directly measured removal rates for kit fox and coyote scats; removal was very high for roads with increased traffic and recommended that future monitoring avoid roads with higher vehicle use. Concerning transferring this technology to other installations, we see the following challenges: 1) unpredictable weather and land access limitations can lead to insufficient sampling, 2) laboratories that can do these analyses need to be identified and likely include a combination of state, federal, university, and private facilities, and 3) experts will need to be identified to conduct quantitative analyses if the necessary expertise is not present within the DoD management team at the implementing installation. The Waits lab is interested in future contract work with DoD to assist in implementation of this technology at other installations.

# 1.0 INTRODUCTION

## 1.1 BACKGROUND

Lands managed by the Department of Defense (DoD) support the greatest densities of species of conservation concern (i.e., endangered, threatened or otherwise at-risk) among lands operated by federal agencies (Stein et al. 2008). Under the provisions of the Endangered Species Act (ESA) and the Sikes Act, the DoD is challenged to protect and provide for the conservation of these species while simultaneously adhering to the military mission. To facilitate these efforts and evaluate the effects of management or training actions, the DoD needs reliable, accurate and cost-effective methods for assessing and monitoring the status of species of concern while minimizing impacts on military training. Specifically, managers need methods for estimating population distribution, abundance, survival, reproduction, movements, and genetic diversity.

Despite the widely recognized need for reliable monitoring data, many vertebrate species are notoriously difficult to monitor because they are wide-ranging, occur in low densities, or are otherwise not amenable to traditional methods. For example, some of the more common approaches (e.g., telemetry, capture-recapture, aerial surveys, point counts) for populations rely on direct visual observation and physical capture of animals, are logistically difficult, and can be expensive and time consuming to implement at large spatial scales over long time frames. Other methods (e.g., scent-station surveys, spotlight counts, scat deposition rate surveys, and activity indices) may be more practical to implement but provide only indices of abundance, neglecting important information on true population size, survival, reproduction and movements (Long et al. 2008). Thus, there is great potential for DoD to benefit (i.e., relaxed restrictions on training and land use, increased cost efficiency, and enhanced species conservation) from implementing the most effective and innovative approaches for monitoring vertebrate populations.

One promising new approach that is becoming more and more prevalent in the scientific community, but has yet to see widespread use by DoD, is based on combining noninvasive genetic sampling (NGS) with demographic parameter estimation techniques. For example, noninvasive genetic sampling can be combined with capture-recapture methods (NGS-CR) to accurately and efficiently evaluate the status of populations (e.g., abundance, survival). A primary advantage of NGS-CR is that populations can be inventoried and monitored by collecting hair, feces or feathers, without the need to disturb, capture, or even see the animals (Taberlet et al. 1999, Beja-Pereira et al. 2009), an important consideration for species of concern. Many recent studies have applied this approach due to logistical considerations or improved cost-benefit (e.g., Prugh et al. 2005, Boulanger et al. 2008, Kendall et al. 2008, Meijer et al. 2008, Marucco et al. 2009, Broseth et al. 2010, Stenglein et al. 2010a). Additionally, under an appropriate spatio-temporal sampling design, NGS can be combined with occupancy modeling (NGS-OM) to efficiently and accurately obtain estimate occupancy parameters (e.g., proportion of area occupied, patterns of local colonization and extinction).

## 1.2 OBJECTIVE OF THE DEMONSTRATION

The primary goal of this project was to demonstrate how noninvasive genetic sampling (NGS) could be efficiently combined with capture-recapture modeling (NGS-CR) to evaluate the status of species of conservation concern and their responses to management or military training and testing activities. We evaluated the efficacy of NGS-CR as a viable, long-term inventory and monitoring approach for species of concern on DoD installations by comparing the cost-benefit with alternative approaches. To facilitate this technology as an option for DoD installations and demonstrate its transferability, we implemented monitoring programs for two species of management interest to DoD: the kit fox (*Vulpes macrotis*), a species of concern for western installations, and Sonoran pronghorn (*Antilocapra americana sonoriensis*), an endangered subspecies that occurs in southern Arizona. For each species we developed a spatio-temporal sampling design for acquiring noninvasive genetic data (via fecal scats) from individuals, genotype samples for individual identification, analyze genotypes with capture-recapture methods to obtain estimates of key population parameters, and develop a protocol for long-term monitoring in the future. For each task, we quantified expenditures and performed a cost-benefit analysis of the approach.

Specific objectives were to: (1) develop and implement spatio-temporal sampling designs for collection of fecal samples for use within a capture-recapture models, (2) quantify variation in estimates of population parameters and perform a power analysis to determine the sampling effort required to achieve desired levels of precision in estimates of population parameters, (3) develop efficient long-term monitoring protocols that integrate an appropriate spatial and temporal sampling design with NGS-CR approaches, (4) compare cost-benefit of monitoring these populations using NGS-CR versus alternative methods, and (5) facilitate transference to other species of concern occurring on DoD installations for which NGS-CR approaches would be a preferred alternative.

NGS-CR approaches generally implement one of two major approaches for collecting samples: (1) standardized transect sampling (Kohn et al. 1999, Prugh et al. 2005, Kendall et al. 2009, De Barba et al. 2010) and (2) targeted sampling in areas where animals congregate to access food or water resources, reproduce, roost, or defecate (Prigioni et al. 2006, Puechmaille and Petit 2007, Rudnick et al. 2008, Robinson et al. 2009, Stenglein et al. 2010a). To maximize transferability of the methods, we chose focal species of concern to DoD that required the application of both standardized (kit foxes) and targeted (pronghorn) sampling approaches.

A secondary goal of this project was to demonstrate how NGS-OM could be employed as an alternative monitoring strategy for species of concern. While NGS-CR methods provide an effective and cost-efficient approach for estimating population demographics, managers may require only information on species occurrence, or may want to monitor the spatial dynamics of populations, both of which can be accomplished through NGS-OM at reduced costs relative to NGS-CR. Additionally, for investigating species-habitat and interspecific interactions, NGS-OM provides a cost-efficient and effective alternative to approaches such as live-capture and radio-telemetry.

To demonstrate NGS-OM techniques, we focused on kit foxes, and their sympatric intraguild predator, coyotes (*Canis latrans*) at DPG. We developed a spatio-temporal sampling design for

acquiring canid scats that would allow us to monitor these species simultaneously. We used genetic analyses to confirm species (De Barba et al. 2014) and dynamic occupancy models to obtain estimates of detection, proportion of area occupied, and dynamic parameters (i.e., local colonization and local extinction), and to evaluate the influence of coyotes and landscape features on the observed patterns of kit fox space-use (MacKenzie et al. 2006). We quantified expenditures and compared the cost of monitoring with NGS-OM to monitoring with NGS-CR or both approaches.

## 1.3 REGULATORY DRIVERS

The DoD, under the Sikes Act, is responsible for conserving and protecting biological resources on its lands. Under the Sikes Act military installations are required to prepare, implement, review, and revise Integrated Natural Resource Management Plans (INRMPs) to provide for conservation of species at risk and other natural resources. Additionally, the ESA requires that federal agencies comply with the provisions of the law by not jeopardizing the persistence of endangered or threatened species. Other DoD guidance related to management of listed species and biological diversity include DoD Instruction 4715.03, Army Regulation 200-3, U.S. Air Force Instruction 32-7064, U.S. Navy Instruction OPNAVINST 5090.1B, and U.S. Marine Corps Order MCO-P5090.2A.

Thus, military installations are tasked with implementing management strategies and creating programs to enhance wildlife conservation while maintaining mission readiness. As a result, DoD installations need monitoring programs that can be used to determine the status of wildlife populations, evaluate management actions influencing population dynamics, and assess changes through time. Consequently, DoD installations benefit from reliable monitor strategies that estimate population distribution, abundance, survival, reproduction, genetic diversity, and movements.

# 2.0 TECHNOLOGY/METHODOLOGY DESCRIPTION

## 2.1 TECHNOLOGY/METHODOLOGY OVERVIEW

*NGS-CR technology description*

Capture-recapture modeling (reviewed by Williams et al. 2002, Amstrup et al. 2005, and Sandercock 2006) has been commonly used to estimate population parameters for wild animals. The theory is based on modeling initial capture and recapture probabilities of populations or individuals as a function of population size, survival, reproduction and movements among populations. The approach was first used to estimate wildlife population abundance by Lincoln (1930) but the approach has been advanced to estimate several other demographic parameters such as survival, reproduction, and movements among spatially separated populations. The basic process involves capturing individuals and marking them in such a way that on subsequent capture occasions, marked individuals can be identified. Using the observed capture histories, demographic parameters can be estimated.

In capture-recapture modeling, the temporal spacing of capture occasions is referred to as the sampling design. One of the most powerful sampling designs, Pollock's robust design (Pollock 1982, Pollock et al. 1990, Kendall et al. 1997), consists of two types of sampling periods. Primary sampling periods are separated by relatively long time intervals (e.g., several months or years), during which it is assumed that individuals in the population may reproduce, die, or move in or out of the study area. Within each primary period are secondary sampling periods separated by relatively short time intervals (e.g., days or weeks), during which the population is assumed to be demographically and geographically closed (i.e., very few or no individuals reproduce, die or move among subpopulations). The robust design allows for estimation of population size at each primary period, survival and temporary emigration between primary periods, and capture probability during secondary periods (Figure 1).

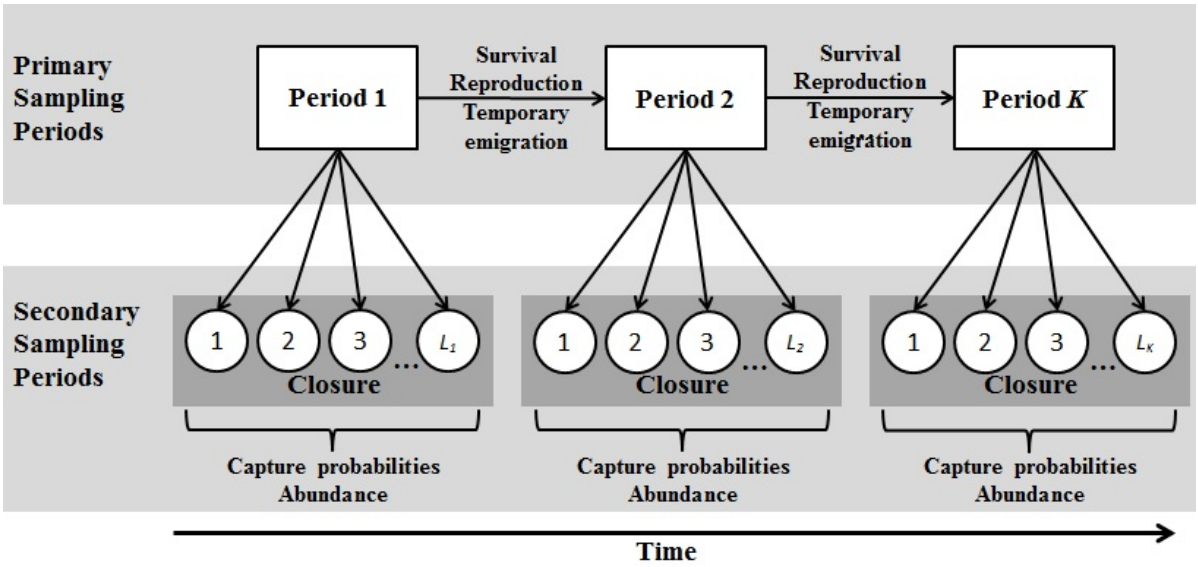



**Figure 1. Pollock's robust sampling design for capture-recapture analyses.**

Recent extensions of the robust design have allowed for partitioning recruitment into gains from reproduction versus immigration (reviewed by Sandercock 2006) and the incorporation of genotyping error when using NGS-CR (Lukacs et al. 2009). Following Lukacs et al. (2009), a joint multinomial likelihood is constructed for an observed encounter history $\mathbf{h} = \{h_1, h_2, \ldots, h_L\}$ with $L$ secondary capture occasions across all primary occasions, where $h_i = 1$ if the genotype is observed, and 0 otherwise. Parameters of this likelihood where $i$ indexes primary periods and $j$ indexes secondary periods include:

$\varphi_i$ = probability of surviving between primary periods and remaining within the population

$\gamma_i^{'}$ = probability of remaining off of the sampling area

$\gamma_i^{''}$ = probability of temporarily leaving the sampling area

$p_{ij}$ = probability of initially observing a genotype (i.e., probability of capture)

$c_{ij}$ = probability of subsequently observing a genotype (i.e., probability of recapture)

$\alpha_i$ = probability that the first observation of a genotype is a correct one

The likelihood for the observed encounter histories is maximized over these parameters providing maximum likelihood estimates. Subsequently, an estimate of population abundance is derived from these maximum likelihood estimates.

Data collection under Pollock's robust design allows the most flexibility to estimate population parameters but also allows for estimation under alternative capture-recapture models such as Cormack-Jolly-Seber and several single-session estimators of abundance (Lukacs and Burnham 2005, Miller et al. 2005, Puechmaille and Petit 2007).

NGS-CR is an attractive and innovative approach because sampling of hair, feces or feathers provides DNA material of free-ranging animals that can be used to identify individuals within the population without having to catch, handle, or even observe them and can be more cost-effective than traditional methods that require live capture (Solberg et al. 2006, Marucco et al. 2009, DeBarba et al. 2010, Stenglein et al. 2010a). If an appropriate temporal and spatial sampling design is employed, recorded "captures" of unique genotypes can be used in capture-recapture models to estimate parameters (i.e., abundance, survival, reproduction, immigration, and emigration) required for population assessment and viability analysis (Lukacs and Burnham 2005, Petit and Valiere 2005; Figure 2).

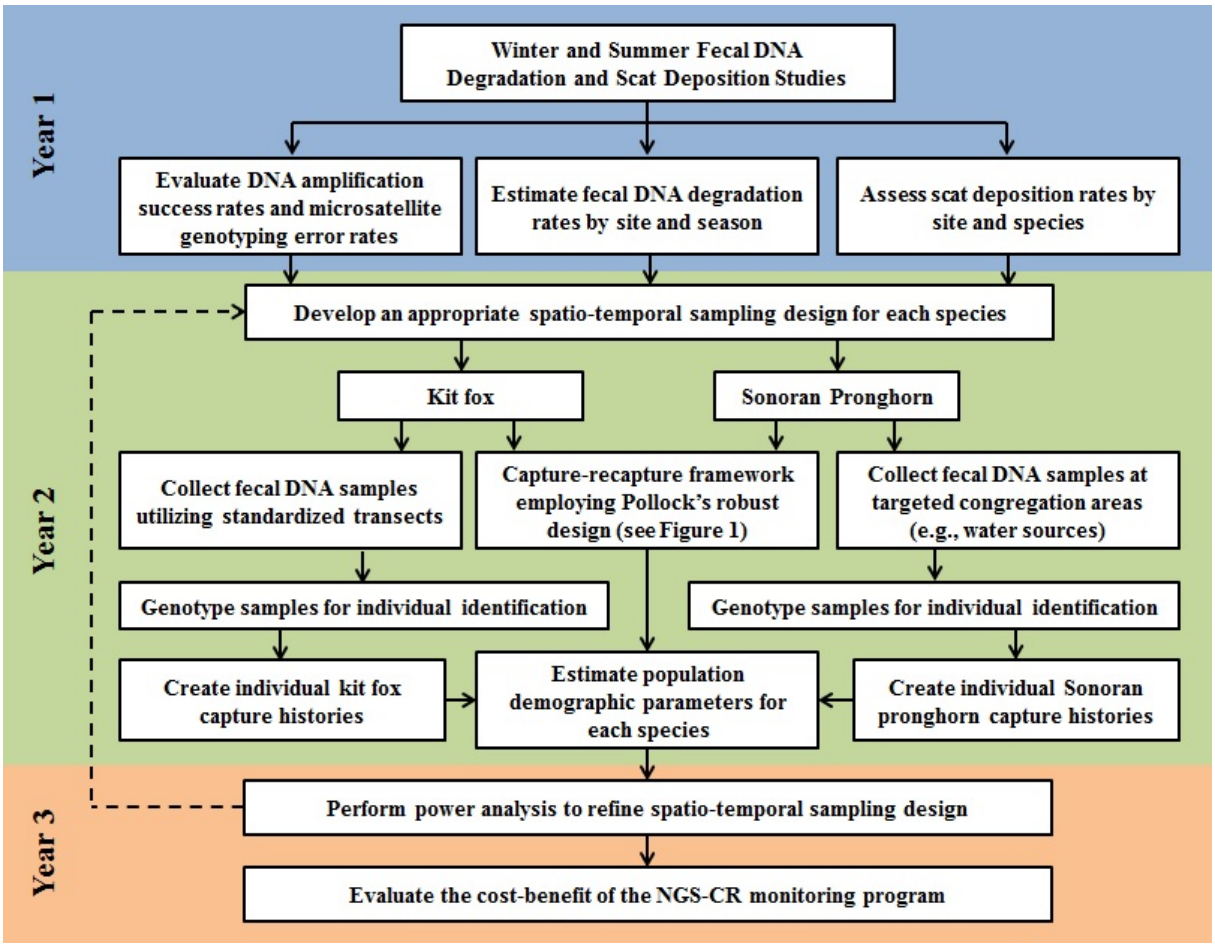



**Figure 2. Flow diagram of the demonstration's technology and methodology.**

*Chronological summary of NGS-CR technique development*

NGS was first introduced in 1992 as a method to obtain genetic samples from rare and elusive brown bears (*Ursus arctos*) in Europe (Höss et al. 1992, Taberlet and Bouvet 1992) and to study social structure in chimpanzees (*Pan troglodytes;* Morin and Woodruff 1992*).* In the last 20 years researchers have demonstrated a variety of important applications for NGS including detection of rare species, population estimation, hybridization analyses, gene flow analyses, mating system studies, predation studies, diet analyses, and forensic applications (Waits and Paetkau 2005, Beja-Pereira et al. 2009). Challenges associated with NGS include fecal DNA degradation, genotyping errors, and contamination (Taberlet et al. 1997, 1999, Waits and Paetkau 2005, Beja-Pereira et al. 2009). Degradation of DNA is influenced by time (i.e., age of a sample; Piggott 2004, Murphy et al. 2007, Santini et al. 2007), environmental factors (e.g., temperature, moisture, substrate, diet; Murphy et al. 2003, 2007, Piggott 2004, Santini et al. 2007), storage (Panasci et al. 2011), and species (Piggott 2004). DNA degradation can result in genotyping errors such as allelic dropout and false alleles (Pompanon et al. 2005), which will result in an over-estimate of population size if not addressed (Waits and Paetkau 2005). Contamination risk is increased in NGS studies due to the low quantity and quality of samples (Pompanon et al. 2005, Waits and Paetkau 2005). As the field has evolved, researchers have

developed methods for addressing these weaknesses and producing accurate data using NGS (Taberlet et al. 1999, Mills et al. 2000, Miller et al. 2002, Paetkau 2003, Broquet and Petit 2004, Waits and Paetkau 2005).

Recently, several studies have shown that NGS-CR can effectively and efficiently monitor vertebrate populations (e.g., Prugh et al. 2005, Solberg et al. 2006, Meijer et al. 2008, Kendall et al. 2009, Marucco et al. 2009, Beja-Pereira et al. 2009, De Barba et al. 2010). While most early applications involved carnivore species, the approach has also been extended to several species of ungulates (e.g., Valiere et al. 2007, Harris et al. 2010, Brinkman et al. 2011) and birds (e.g., Rudnick et al. 2005, Regnaut et al. 2006, Jacob et al. 2010). The DNA genotyping technology and statistical framework are sufficiently mature (Lukacs and Burnham 2005, Petit and Valiere 2005, Waits and Paetkau 2005, Beja-Pereira et al. 2009, Luikart et al. 2010) to proceed with a demonstration of its utility and assess the cost and performance for DoD applications.

Fecal DNA surveys have been used to detect the presence of the San Joaquin kit fox (*V. m. mutica*) in California (Smith et al. 2005) and molecular methods have been developed to conduct sex identification (Ortega et al. 2004) and individual identification (Smith et al. 2006) of this subspecies. In addition, several studies have successfully used NGS-CR for monitoring other canids (e.g., arctic fox [*V. lagopus*], Meijer et al. 2008; coyotes, Prugh et al. 2005; and wolves [*C. lupus*], Marucco et al. 2009, Stenglein et al. 2010a). While application of NGS-CR to ungulates has generally lagged behind application to carnivores, studies have successfully implemented NGS-CR for red deer (*Cervus elaphus*; Valiere et al. 2007), argali sheep (*Ovis ammon polii*; Harris et al. 2010), and Sitka black-tailed deer (*Odocoileus hemionus sitkensis*; Brinkman et al. 2011). Also, PI Waits has experience using NGS techniques on pronghorn (*A. americana*) populations in Montana to evaluate genetic diversity and mating system (Dunn et al. 2011).

*NGS-OM technology description*

Species tend to be patchily distributed in association with required resources (e.g., forage, shelter, mates, etc.; Vandermeer 1972). Species distributions can be restricted by competition and/or predation. Knowledge of how habitat and interspecific interactions drive the space-use by species of conservation concern can improve management policies. This information is often difficult and expensive to obtain, particularly for imperiled species that may already be in decline and for which rapid assessments would be preferred (MacKenzie et al. 2006). Patterns of species occurrence are often derived from presence-absence data. Noninvasive sampling strategies may increase detection rates for rare or elusive species, reducing costs and minimizing impacts on species (e.g., Long et al. 2011). One limitation of noninvasive surveys is the potential for misidentification of sign, particularly when sympatric species produce similar sign (e.g., tracks, scat). Furthermore, when investigations of habitat relationships are performed with traditional approaches, such as logistic regression, inclusion of false absences (i.e., a species is present but goes undetected) tend to biased results (MacKenzie et al. 2006). Occupancy modeling utilizes information from repeat surveys to account for imperfect detection and produces unbiased parameter estimates (MacKenzie et al. 2002, 2003, 2006). Unlike NGS-CR, the unit of analysis in occupancy studies is the survey site (or patch), as opposed to the individual. Consequently, patterns of occurrence can be modeled as a function of patch characteristics, such as habitat or

landscape features. One assumption of occupancy modeling is no misidentification of species (or sign) and even small amounts of misidentification can severely bias inferences (MacKenzie et al. 2006). By employing NGS-OM approaches, unambiguous genetic species identification can be used to ensure accurate species identification. Because occupancy modeling focuses on the species, analyses do not require individual identification, reducing costs associated with genetic analyses when compared to NGS-CR.

Likelihood-based occupancy modeling has seen extensive use over the past decade to effectively assess species occurrence and distribution (e.g., Nichols et al. 2008, Long et al. 2011, Jones 2011). Covariate data, such as landscape and habitat features, can be incorporated into occupancy models to assess the influence of environmental variables on occupancy (MacKenzie et al. 2006, Long et al. 2011). Recent advances in multi-species occupancy models (i.e., co-occurrence models; Richmond et al. 2010) and dynamic occupancy models (i.e., multi-season models or robust design occupancy models; MacKenzie et al. 2003) have extended the utility of this monitoring strategy, and allow practitioners to investigate species interactions and dynamic processes (i.e., local colonization and extinction) that drive observed states of occupancy.

Like capture-recapture modeling, replication required to estimate probability of detection for occupancy modeling may be accomplished by repeating surveys over time (i.e., temporal replication). Alternatively, spatially disparate surveys (i.e., spatial replication) within a site, or patch, can be used to estimate detection probability. Using spatial replication in lieu of temporal replication can maximize spatial coverage and minimize field costs. Still, sampling spatial replicates without replacement can bias parameter estimates (Kendall and White 2009); sampling with replacement may be impractical for noninvasive genetic sampling when all surveys are conducted within a single site visit and searcher efficiency is high (i.e., all or most of the scats present are detected). Alternatively, sampling spatial replicates without replacement does not bias results if occupancy is constant for each replicate (Guillera-Arroita 2011) or if the target species is highly mobile (Kendall and White 2009, Harris et al. 2014), as is the case with kit foxes and coyotes at our study site.

While single-season occupancy modeling has become a common monitoring strategy for wildlife populations, these models only provide estimates of detection and proportion of area occupied. Dynamic occupancy models allow practitioners to formally assess patterns of colonization and local extinction that drive static occupancy states (MacKenzie et al. 2003, 2006). Like Pollock's robust design for capture-recapture analyses (Figure 1), dynamic occupancy models consist of both primary periods (commonly referred to as seasons in the occupancy literature) and secondary periods (Figure 3). Sampling sites, or patches, are assumed to be closed to changes in occupancy state within primary sampling periods; changes in occupancy may occur between primary sampling periods.

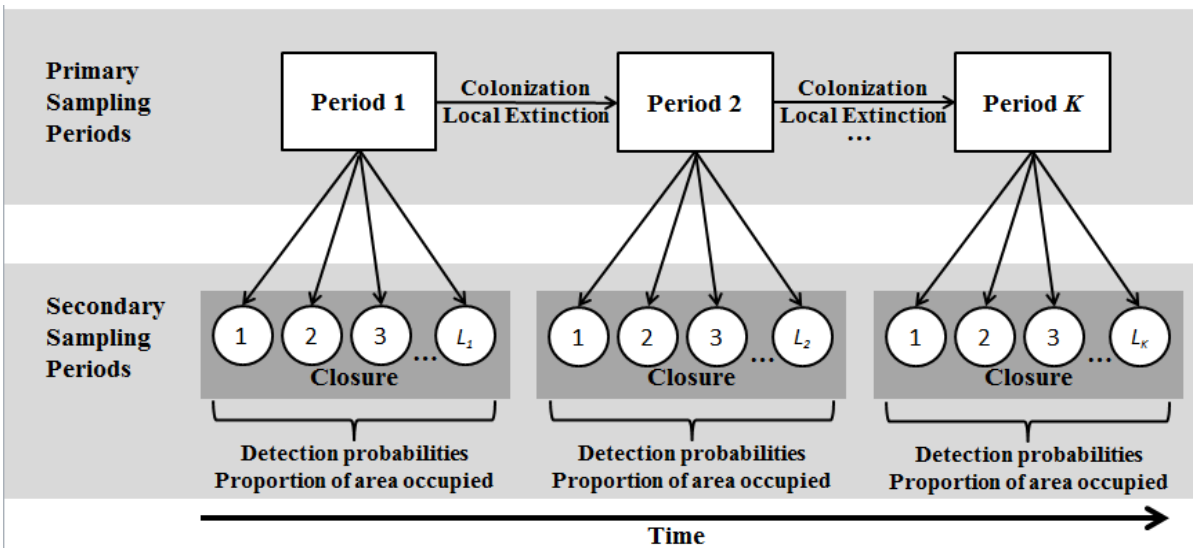



**Figure 3. Dynamic (multi-season) sampling design for occupancy modeling analyses.**

Occupancy modeling can be extended to test for evidence of competitive exclusion of a subordinate species (e.g., kit fox) by a dominant species (e.g., coyote). Recent advancements in co-occurrence modeling (i.e., multi-species occupancy models) have resulted in a model that is stable when covariate data is included, providing a tool to investigate competitive exclusion while accounting for the influence of habitat and landscape features (Richmond et al. 2010). This is particularly relevant to competition between kit foxes and coyotes at our demonstration site, where it had been postulated that increased water availability has facilitated increased coyote occurrence (Arjo et al. 2007).

*Expected applications*

This project will benefit our demonstration installations and will provide information that can be employed across DoD installations. This project will directly benefit Dugway Proving Ground (DPG) and neighboring Hill Air Force Range by providing information needed to manage kit foxes and evaluate effects of military training and management actions on kit fox populations. These efforts will be critical to proactively preventing kit foxes from being listed under the ESA and, thus, preclude subsequent restrictions imposed by listed species on DoD lands. For Sonoran pronghorn, there are already substantial financial and training costs to DoD because of their ESA endangered status (McCullough 2005). To be downlisted, it will be necessary to have reliable monitoring information from the re-established populations of Sonoran pronghorn. This methodology can provide estimates of population size, trend, reproduction, survival rates, and genetic diversity and connectivity.

In addition to directly benefiting the focal species and installations of this project, the we see potential for monitoring other species of concern such as San Joaquin kit foxes at Camp Roberts and Fort Hunter Liggett, kit foxes at White Sands Missile Range and Fort Bliss, swift foxes (*V. velox*) at Piñon Canyon Maneuvering Site, Island gray foxes (*Urocyon littoralis*) at San Clemente and San Nicolas Island Naval Reservations, Florida panthers (*Puma concolor coryi*) at Camp Blanding and Avon Park Range, and gray wolves at Camp Ripley and Fort McCoy. The

standardized transect sampling approach could also be helpful for monitoring Florida black bears (*Ursus americanus floridanus*), which are currently present on four military instillations. The concentrated sampling approach would likely be effective for monitoring cave roosting bat species (Indiana bat [*Myotis sodalist*], gray bat [*M. grisescens*]) that are currently species of concern at 16 installations.

## 2.2 TECHNOLOGY/METHODOLOGY DEVELOPMENT

To inform our sampling design, it was necessary to understand (1) the rate at which scat accumulated for each target species and (2) how quickly fecal DNA degraded in each local climate. Scat accumulation rates influence the number of samples available for collection during surveys and was expected to directly affect the temporal sampling design for capture-recapture analyses. DNA degradation rates influence fecal DNA amplification success rates and genotyping error rates, which interact to determine success rates for both species identification and individual identification. DNA degradation rates also determine how long after deposition an individual can be confidently identified, restricting the age of scats that could be successfully incorporated into NGS-CR analyses. To inform sampling in year 2 and 3, we evaluated patterns of scat deposition and DNA degradation for each target species during year 1. Specifically, this data was used to determine (1) if it is necessary to remove scats from sampling transects before the first secondary sampling session in each primary sampling period and (2) the optimal time period between secondary sampling sessions.

For carnivores, noninvasive surveys (Long et al. 2008, Kelly et al. 2012) are appealing because they are simple, cost-efficient, and facilitate multi-species monitoring (Gompper et al. 2006). For many elusive or rare species, scats are often the most conspicuous indication of their presence and therefore noninvasive scat surveys are a widely used means of monitoring populations (e.g., Prugh and Ritland 2005, Gompper et al. 2006, Harrington et al. 2010, Long et al. 2011). Carnivore scat surveys typically involve surveying along roads (Schauster et al. 2002, Schwalm et al. 2012, Dempsey et al. 2014) or trails (Kohn et al. 1999, Gompper et al. 2006) at set sampling intervals. Correct inferences from scat surveys depend on accurate species identification and assume the scat persistence (or inversely removal from natural decay and/or disturbance) is constant among survey sites and target species. Both scat misclassifications and inequitable removal rates could bias results and potentially reduce the effectiveness of management strategies (Marucco et al. 2008, Harrington et al. 2010, Lonsinger et al. 2015b, Lonsinger et al. 2016). To improve future sampling, we used kit foxes and coyotes at DPG as model species to (1) assess the accuracy of field based identification (i.e., based on physical appearance, odor, etc.) and evaluate the success of alternative nonparametric statistical classification techniques, and (2) to examine factors influencing variation in carnivore scat removal. This data was then used to inform recommendations for future study designs that could improve efficiency of long-term monitoring of carnivores.

*Kit fox sample accumulation and DNA degradation*

We developed a model for combining sample accumulation and DNA degradation to identify the most efficient (i.e., minimal cost per successful sample) temporal design for NGS-CR analyses

(Figure 4). We evaluated sample accumulation and fecal DNA degradation for both kit foxes and coyotes simultaneously. Although this demonstration pertains only to kit foxes, our analyses for kit foxes and coyotes were accomplished as a single analysis, increasing our sample size and improving our precision. Consequently, we present the results for both kit foxes and coyotes. Detailed results associated with the evaluation of kit fox and coyote sample accumulation and DNA degradation rates are published in Lonsinger et al. (2015a).

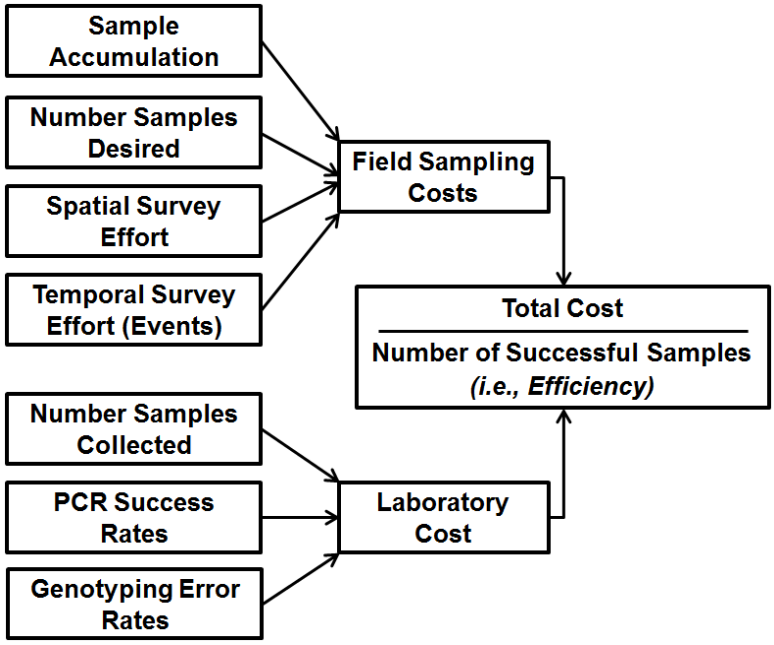



**Figure 4. Conceptual diagram showing the major components required to balance field and laboratory efficiency for optimization of noninvasive genetic sampling for capture-recapture analyses (source: Lonsinger et al. 2015a).**

Scat accumulation surveys in which transects are cleared and surveyed ~14 days later are commonly used to estimate relative abundances of canids (Gese 2001, Schauster et al. 2002). Using this approach, we estimated scat accumulation rates for kit foxes and coyotes by clearing and surveying transects for scats between September 2010 and July 2012. Surveys were conducted during three seasons: winter, summer, and spring. Fifteen 5 km transects along dirt or gravel roads were cleared and surveyed ~14 days later (mean = 13.9 ± 0.51 SD, range = 13–16). Each 5 km transect was surveyed during two summers (2010, 2011), two springs (2011, 2012) and one winter (2011). To expand the spatial coverage and ensure that standardized accumulation rates (scats/km/day) were similar between sampling intervals of different durations, we evaluated accumulation along eight shorter transects during one summer (2012), using a random length (mean = 2.6 ± 0.85 SD, range = 1–3.5 km) and surveying seven days after clearing. We determined species for each carnivore scat detected during accumulation surveys based on overall appearance, size and shape (*sensu* Kozlowski et al. 2012).

Rates of scat accumulation were higher for coyotes (mean = 0.076 scats/km/day ± 0.009 SE) than kit foxes (mean = 0.029 scats/km/day ± 0.007 SE) across seasons (Figure 5). We employed a generalized linear model to test effects of season and species on scat accumulation (O'Hara and

Kotze 2010). We used a likelihood ratio test to compare models with and without interactions. Species had a significant effect on scat accumulation when controlling for season (contrast, z = -9.09, $P < 0.001$; Table 1). Season contrasts controlling for species indicated that spring accumulation rates were significantly different from summer (contrast, z = 5.99, $P < 0.001$) and winter (contrast, z = -3.16, $P = 0.002$), but that summer and winter differed only marginally (contrast, z = 1.89, $P = 0.059$; Table 1; Figure 5).

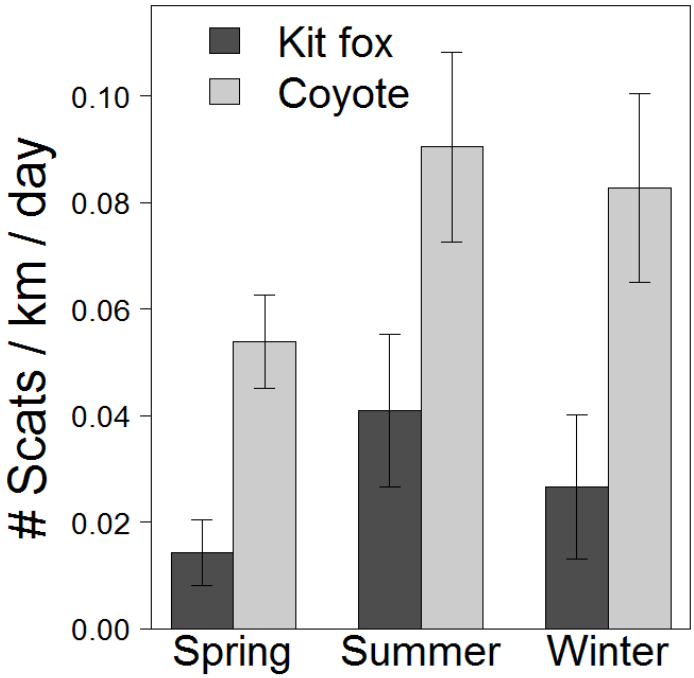



**Figure 5. Mean scat accumulation rates ± SE for kit fox (*Vulpes macrotis*) and coyote (*Canis latrans*) at Dugway Proving Ground, Utah, collected from September 2010 to July 2012 (source: Lonsinger et al. 2015a).**

Fecal DNA degradation was assessed at DPG for kit foxes and coyotes during two seasons, winter (2012) and summer (2012), corresponding to proposed field sampling seasons. We placed 20 fresh scats/species/season in the field under natural field conditions and protected them from disturbance with a wire mesh (25mm openings; 0.7 gauge wire) covered frame. We collected fecal DNA samples from each scat at days 1, 3, 7, 14, 21, 56 and 112, or until the scat was fully utilized. We added a day 5 time point during summer to provide greater resolution, as a recent study detected a significant decline in coyote fecal DNA quality as early as five days post-deposition (Panasci et al. 2011). A wind event during winter buried experimental plots after day 21, so day 56 and 112 time points were only available for summer. Fecal DNA samples were collected from the side of each scat following procedures of Stenglein et al. (2010a) and samples were stored in 1.4 ml of DETs buffer (Seutin et al. 1991). Due to natural variability in scat sizes, some smaller scats were fully utilized before completion of all time points, resulting in reduced sample sizes at later time points. To maintain more equitable sample sizes among time points during summer, we placed three additional scats for each species out at the start of the degradation study and sampled these scats in place of fully utilized scats at later time points.

For fecal DNA samples, we evaluated temporal changes in amplification success rates for mitochondrial (mtDNA) and nuclear (nDNA) DNA, and genotyping errors for nDNA. Across species and seasons, mtDNA amplification success was ≥95% through day 21. Kit fox nDNA amplification success was ≥70% through day 21 across seasons (Figure 6). Coyote nDNA success was ≥70% through day 21 in winter, but declined to <50% by day 7 in summer (Figure 6). Overall genotyping error rates varied between species (Figure 7); across seasons and sampling periods, overall allelic dropout (ADO) was lower for kit foxes (18%) than coyotes (25%), while overall false allele (FA) rate was slightly higher for kit foxes (5%) than coyotes (2%). Winter samples of both species had lower genotyping error rates on average than summer samples.

**Table 1. Generalized linear model and contrast analysis results with standard errors (SE) and lower (LL) and upper (UL) 95% confidence bounds for scat accumulation samples collected from September 2012 to July 2012 at Dugway Proving Ground, Utah. Species levels include coyote (*Canis latrans*) and kit fox (*Vulpes macrotis*). Season levels include spring, summer and winter. Significant (*) *p*-values for z statistic evaluated at α = 0.05 (source: Lonsinger et al. 2015a).**

| | Estimate | SE | z-value | *P*-value | LL | UL |
|---|---|---|---|---|---|---|
| *Model Parameters* | | | | | | |
| (Intercept) | -3.07 | 0.243 | -12.37 | <0.001* | -3.52 | -2.56 |
| Summer | 0.66 | 0.277 | 2.38 | 0.019* | 0.13 | 1.22 |
| Winter | 0.47 | 0.349 | 1.36 | 0.177 | -0.23 | 1.16 |
| Kit fox | -0.97 | 0.253 | -3.83 | <0.001* | -1.49 | -0.49 |
| *Contrasts* | | | | | | |
| Coyote vs. Kit fox | -1.08 | 0.119 | -9.09 | <0.001* | -1.32 | -0.85 |
| Summer vs. Winter | 0.26 | 0.137 | 1.89 | 0.059 | -0.01 | 0.53 |
| Summer vs. Spring | 0.79 | 0.131 | 5.99 | <0.001* | 0.53 | 1.04 |
| Spring vs. Winter | -0.53 | 0.167 | -3.16 | 0.002* | -0.85 | -0.19 |

We evaluated PCR success, FA and ADO as binary response variables with mixed-effects logistic regression models to assess DNA degradation rates, with sample included as a random effect to resolve pseudoreplication effects. We included time, DNA type (mtDNA vs. nDNA), species, season, and locus length as fixed effects in the model for PCR success (Table 2). We excluded DNA type from models for FA and ADO as these pertain only to nDNA. We categorized nDNA locus lengths based on the mid-length of alleles per locus by species (range:

90–275 bp). All of the main effects significantly influenced PCR success (Table 2). Mitochondrial DNA had higher success than nDNA and success for both DNA types decreased over time (Figure 8). Locus length significantly influenced nDNA PCR success, with longer loci having lower success (Figure 8). PCR success was significantly influenced by season, with higher success in winter than summer. Significant interactions among fixed effects reveal the complex nature of DNA degradation (Table 2). PCR success for mtDNA and nDNA declined more slowly in winter than summer and nDNA success declined more precipitously for longer loci than shorter loci (Figure 8). Significant interactions were detected between species and both time and locus length (Table 2). Model results for ADO were influenced by a significant interaction between time and species, while model results for FA were influenced by significant interactions of time with season and species, and locus length with species (Table 2). Model-predicted cumulative genotyping error rates (combined ADO and FA rates across loci and intervals) were lower for kit foxes (winter mean = 20.9% ± 0.6% SE; summer mean = 25.1% ± 0.6% SE) than coyotes (winter mean = 31.5% ± 0.6% SE; summer mean = 37.4% ± 0.5% SE) and higher in summer than winter for both species.

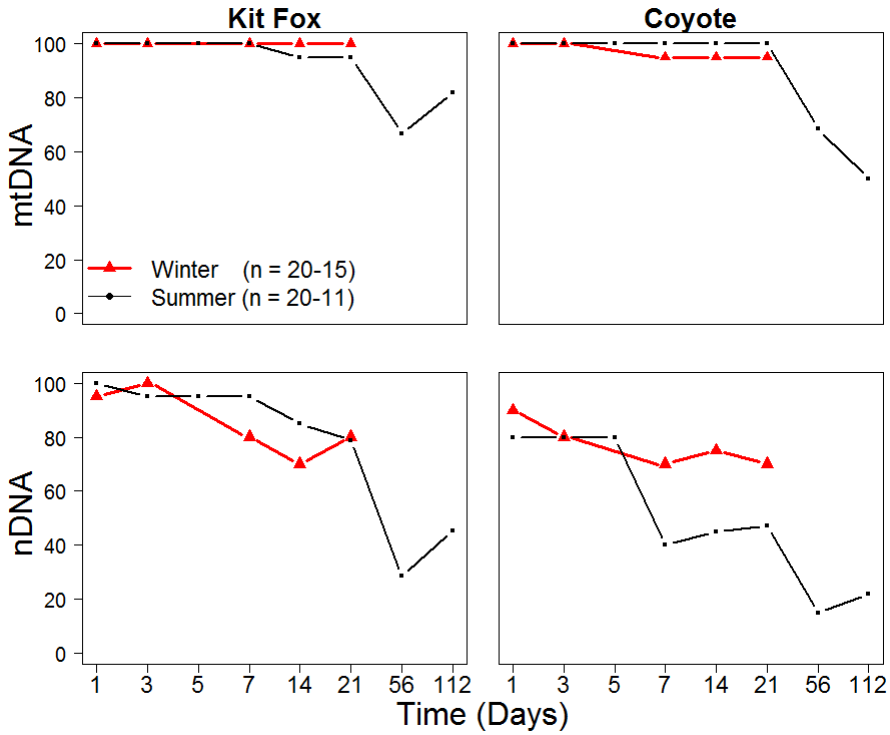



**Figure 6. Observed percent PCR success for mitochondrial (mtDNA) and nuclear (nDNA) DNA for kit fox (*Vulpes macrotis*) and coyote (*Canis latrans*) fecal DNA samples collected in 2012 during winter and summer. Percent PCR success for mtDNA is presented as the proportion of samples identified to species across each time point and season. Percent PCR success for nDNA is presented as the proportion of samples with successful amplification at ≥50% of the loci for each time point and species.**

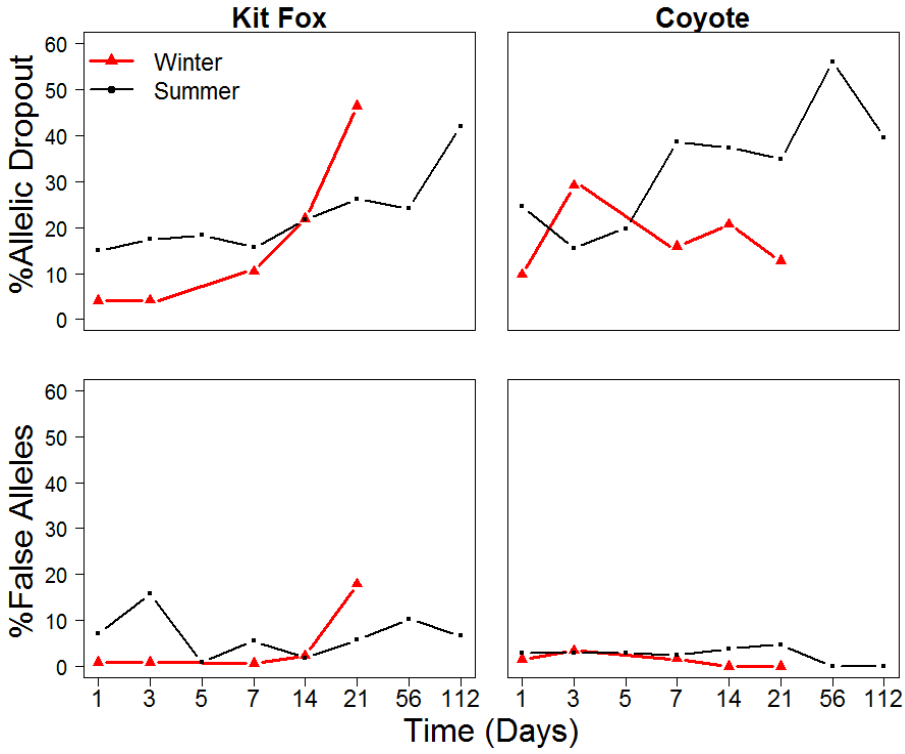



**Figure 7. Observed nuclear DNA genotyping error rates (i.e., allelic dropout and false alleles) for kit fox (*Vulpes macrotis*) and coyote (*Canis latrans*) fecal DNA samples collected in 2012 during winter and summer.**

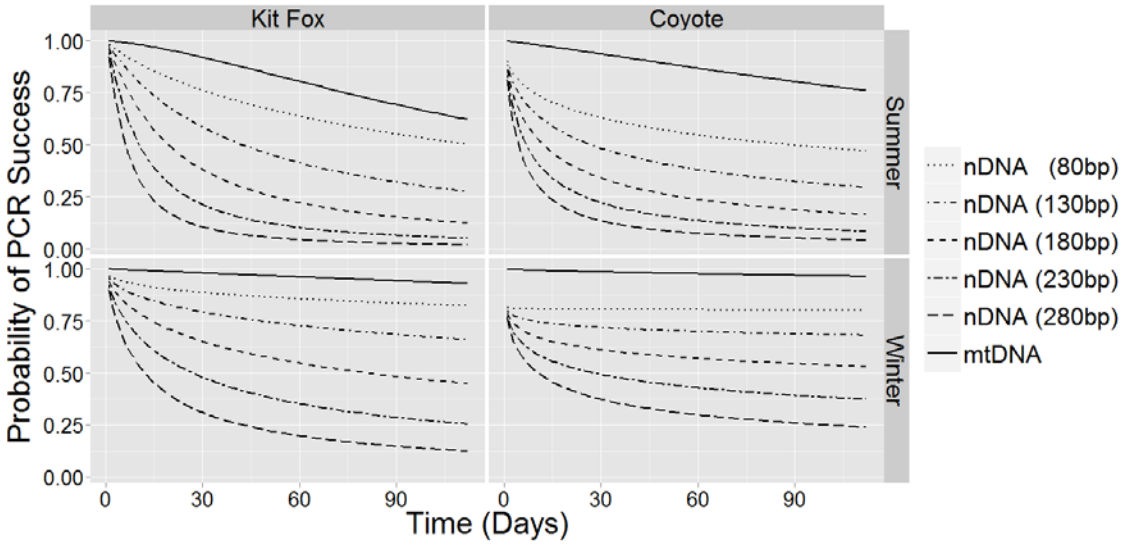



**Figure 8. Mixed-effects logistic regression model results for PCR success for kit fox (*Vulpes macrotis*) and coyote (*Canis latrans*) fecal DNA samples collected in 2012 during winter and summer (source: Lonsinger et al. 2015a).**

Our goal was to optimize a NGS temporal design that could be employed within a capture-recapture framework for kit foxes and coyotes, while maintaining ≤2% probability of error in the final dataset. To this end, we derived a total cost per successful sample (i.e., sample that achieves a consensus genotype for individual identification) at sampling intervals from 1 to 56 days. Field costs accounted for variation in sample accumulation rates between species and sampling effort (Figure 4). Laboratory costs considered both amplification success and genotyping error rates (Figure 4), with the number of replicates required to reduce errors in the final dataset increasing as genotyping errors increased.

**Table 2. Mixed-effects logistic regression model results for PCR success, allelic dropout and false alleles for kit fox (*Vulpes macrotis*) and coyote (*Canis latrans*) fecal DNA samples collected in 2012 during winter and summer at Dugway Proving Ground, Utah. Reported Chi-square test statistics and *P*-values were generated with Type III tests of fixed effects. Significance (*) was evaluated at α = 0.05. Time was log-transformed days since the scat was placed in the field. DNA types included mitochondrial and nuclear DNA (source: Lonsinger et al. 2015a).**

| | PCR success | | Allelic dropout | | False alleles | |
|---|---|---|---|---|---|---|
| Fixed effect | Chi-square | *P*-value | Chi-square | *P*-value | Chi-square | *P*-value |
| Time | 4.93 | 0.0263* | 0.80 | 0.3706 | 0.09 | 0.7678 |
| DNA type | 224.03 | <0.0001* | -- | -- | -- | -- |
| LL | 8.73 | 0.0031* | 0.03 | 0.8661 | 1.26 | 0.2614 |
| Season | 4.02 | 0.0449* | 4.11 | 0.0427* | 0.93 | 0.3337 |
| Species | 25.90 | <0.0001* | 0.64 | 0.4237 | 7.95 | 0.0048* |
| Time × Season | 42.02 | <0.0001* | 0.28 | 0.5966 | 5.91 | 0.0150* |
| Time × Species | 24.15 | <0.0001* | 4.09 | 0.0432* | 4.94 | 0.0262* |
| Time × LL | 13.38 | 0.0003* | 1.03 | 0.3100 | 0.04 | 0.8386 |
| LL × Season | 1.57 | 0.2100 | 1.22 | 0.2699 | 0.15 | 0.7020 |
| LL × Species | 8.36 | 0.0038* | 1.57 | 0.2098 | 10.16 | 0.0014 |

The number of sampling events necessary to obtain desired sample sizes was initially high due to the low number of samples accumulating over shorter intervals, but declined precipitously (Figure 9). The number of sampling events required was typically higher for species (i.e., kit foxes) and seasons (i.e., winter) characterized by relatively lower sample accumulation rates (Figure 9). Overall cost per successful sample showed a similar pattern across species and seasons, but with differences in the magnitude and timing of changes (Figure 9). Costs per successful sample declined as the number of required sampling events reduced field costs, until

genotyping errors were sufficiently high to require additional replicates, increasing laboratory costs (Figure 9). Sharp increases in cost associated with additional replicates occurred at a shorter interval for kit foxes (35 days) than coyotes (50 days) in winter. In summer, sharp increases in cost associated with additional replicates occurred at the same interval (17 days) for both species. When surveying species simultaneously, overall cost per successful sample was reduced (Figure 9c) for each species, due to reduced field costs for each species individually. Average annual cost per successful sample suggested that a temporal sampling frame of ~14 days would reduce costs for each species and allow species to be monitored simultaneously (Figure 9). These results suggest that when conducting repeated surveys for capture-recapture analyses, overall cost-efficiency for NGS-CR may be improved with a temporal design that balance drivers of field and laboratory costs.

This study presents a conceptual model for optimizing NGS-CR sampling, which can be extended to any species or system where estimates of sample accumulation (e.g., hair snaring rate, scat accumulation rate) and DNA degradation rates can be quantified. We demonstrate that this novel optimization approach can effectively reduce costs of NGS monitoring programs. By initiating a pilot study to evaluate sample accumulation and DNA degradation rates, NGS-CR monitoring costs can be minimized, allowing monitoring to occur over larger spatial extents and at higher temporal resolutions than would be possible otherwise. Differences observed in sample accumulation and DNA degradation rates between species and across seasons, at the same study site, reiterate the importance of pilot studies for effectively implementing NGS (Taberlet et al. 1999; Waits and Paetkau 2005).

*Kit fox scat identification*

Scats of sympatric carnivores can be difficult to differentiate and field based identification can be misleading. We evaluated the success of field based species identification for scats of two sympatric carnivores, kit foxes and coyotes. Additionally, we examined the classification success rates of two nonparametric statistical classification schemes, *k*-nearest neighbors (KNN) and classification trees. Although this demonstration pertains only to kit foxes, our analysis for kit foxes inherently includes coyotes (as well as other less abundant species) which co-occur with kit foxes and produce scats of similar characteristics. Consequently, we present the results for both kit foxes and coyotes here. Detailed results are published in Lonsinger et al. (2015b).

We conducted surveys for carnivore scats in winter 2013 and summer 2013 along transects that followed two-track or gravel roads at DPG. We surveyed 30 transects (5 km each) three (summer) to four (winter) times per season. Additionally, we surveyed 240 shorter (500 m each) random transects once per season. For each carnivore scat encountered during surveys, we determined field identification by inspecting the scat's morphology including color, odor, overall size, and physical appearance (*sensu* Kozlowski et al. 2012). We then collected a fecal DNA sample (~0.7 mL) from the side of the scat (Stenglein et al. 2010a), which was stored in 1.4 ml of DETs buffer (Seutin et al. 1991). For a subset of scats sampled, we measured the diameter at widest point and total length with a sterile digital caliper (resolution = 0.1 mm; Mitutoyo America Corporation, Aurora, IL) and recorded the number of disjoint segments, prior to fecal DNA sample collection. We used mtDNA species identification tests (molecular ID; De Barba et al. 2014) to unambiguously determine the species for each scat and calculated the proportion of

samples that were misclassified (hereafter misclassification rate) based on field identification. We subsequently explored the ability of KNN and classification tree analyses to classify scats based on morphometric measurements. We compared the performance of KNN and classification tree models to one another and to field identification based on the misclassification rate.

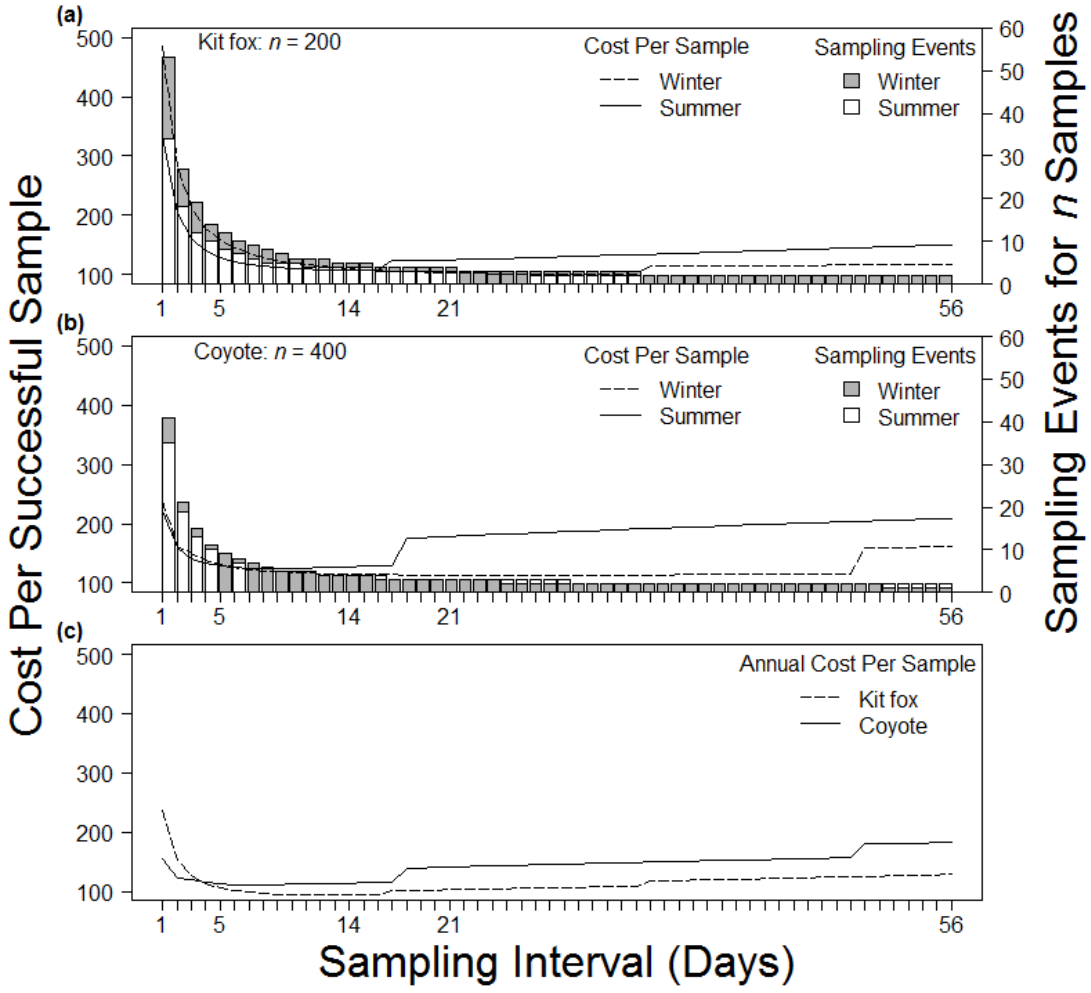



**Figure 9. Evaluation of cost ($) per successful fecal DNA sample and number of sampling events required to obtain (a) *n* = 200 kit fox (*Vulpes macrotis*) and (b) *n* = 400 coyotes (*Canis latrans*) samples from surveying 150 km of transects at Dugway Proving Ground, Utah, for a range of sampling intervals in winter and summer. Sampling intervals represent the days between an initial clear and subsequent survey or between surveys. The average annual cost for surveying each species (c) is reduced when the two sympatric species are surveyed simultaneously (source: Lonsinger et al. 2015a).**

We collected 1,680 (winter: n = 602; summer: n = 1,078) carnivore scats, and field identification and morphometric measurements were available for 1,498. We confirmed species with molecular identification for 1,203 scats. We removed those samples that failed to amplify (285) or were mixed (10) from subsequent analyses. Based on field identification, 70% (848) and 29% (345) of the scats were classified as coyote and kit fox, respectively. The remaining 1% (10) were

classified as red fox (8; *V. vulpes*) or bobcat (2; *Lynx rufus*). Using molecular identification, we confirmed 72% (865) and 24% (293) of the scats as coyote and kit fox, respectively, with <4% confirmed as bobcat (29), red fox (9), domestic dog (6), or cougar (1; *Puma concolor*). The overall misclassification rate, or proportion of samples that were classified as a species different from that confirmed by molecular identification, was 12.2% (Table 3). Of the scats classified as coyote with field identification, 7.1% (60) were misclassified (Table 3). Among scats classified as kit fox with field identification, 22.9% (79) were misclassified (Table 3).

**Table 3. Number of scat samples collected in western Utah, during the winter and summer of 2013 that were classified to species based on field identification and molecular identification. The gray diagonal represents the number of samples correctly classified based on field identification. The misclassification rate was the proportion of samples identified by field identification to a species that was in disagreement with molecular identification (source: Lonsinger et al. 2015b).**

| | | Field identification | | | | | |
|---|---|---|---|---|---|---|---|
| | | Coyote | Kit fox | Bobcat | Dog | Red fox | Cougar |
| | *n* = | 848 | 345 | 2 | 0 | 8 | 0 |
| Molecular identification | Coyote | 788 | 69 | 0 | 0 | 8 | 0 |
| | Kit fox | 27 | 266 | 0 | 0 | 0 | 0 |
| | Bobcat | 23 | 4 | 2 | 0 | 0 | 0 |
| | Dog | 6 | 0 | 0 | 0 | 0 | 0 |
| | Red fox | 3 | 6 | 0 | 0 | 0 | 0 |
| | Cougar | 1 | 0 | 0 | 0 | 0 | 0 |
| Number misclassified | | 60 | 79 | 0 | 0 | 8 | 0 |
| Misclassification rate | | 7.1% | 22.9% | 0.0% | | 100.0% | |

The KNN analysis resulted in overall mean misclassification rates from 11.7% to 16.6% with $k$ = 3 achieving the lowest mean misclassification rate (Figure 10). Mean misclassification rates for coyotes ranged from 12.4% to 18.4% with the lowest mean misclassification at $k$ = 3 (Figure 10), while kit fox misclassifications were lower, ranging from 8.1% to 13.2% with the lowest value at $k$ = 7 (Figure 10). At the optimal $k$ values, the overall mean misclassification rate was reduced, coyote misclassifications increased, and kit fox misclassifications decreased substantially, relative to field identification (Table 3).

Classification tree analyses for kit foxes and coyotes resulted in a decision tree with 4 splits and 5 terminal nodes (Figure 11). Diameter had the highest importance (67/100) followed by length (30/100); segments had little importance (3/100). Misclassification rates produced by the classification tree analysis were lower overall (7.5%) and for coyotes (7.2%), but were higher for kit foxes (8.5%) than those produced by the KNN analysis (Table 4). The classification tree produced a misclassification rate for coyotes similar to field identification (7.1%), but overall misclassification and kit fox misclassification were substantially lower than those from field identification (Table 4).

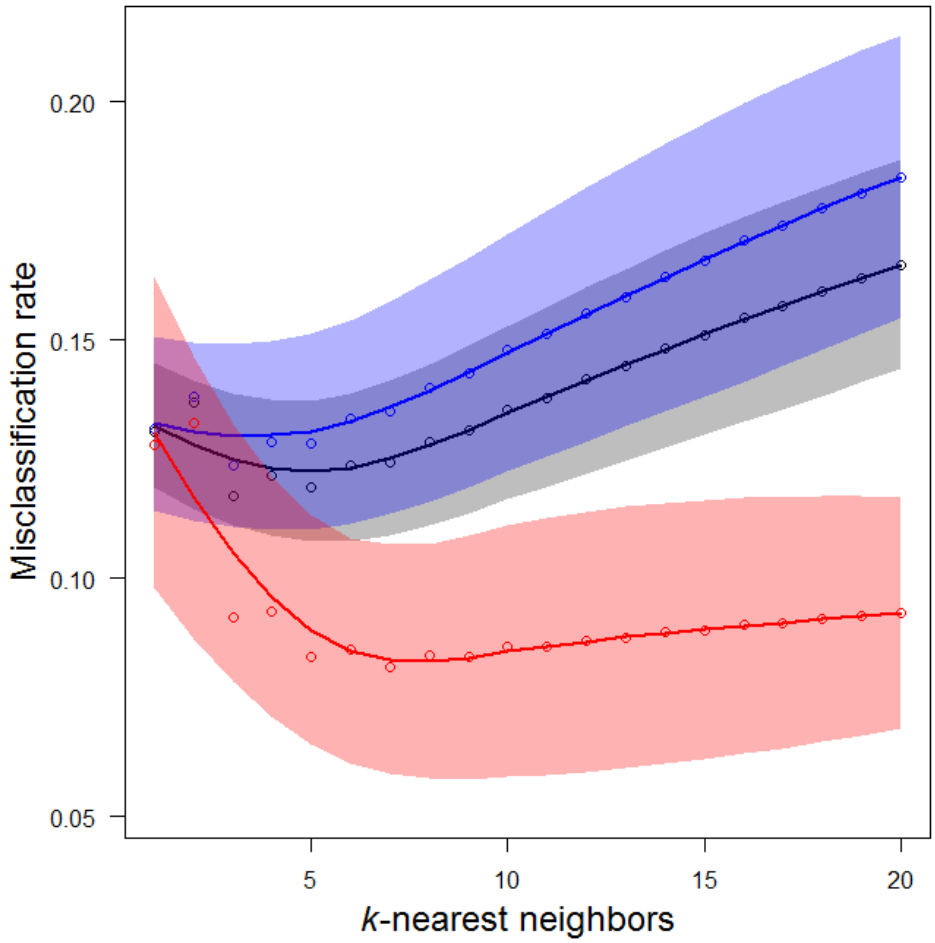



**Figure 10. Mean misclassification rate (±1 SD; bands) for scats of coyotes (blue), kit foxes (red), and overall (black) evaluated at 1–20 *k*-nearest neighbors. The minimum mean misclassification rate was achieved for coyotes (12.4%) at k = 3, for kit foxes (8.1%) at k = 7, and overall (11.7%) at *k* = 3. Scat samples were collected in Tooele County, Utah (USA) in the winter and summer of 2013 (source: Lonsinger et al. 2015b).**

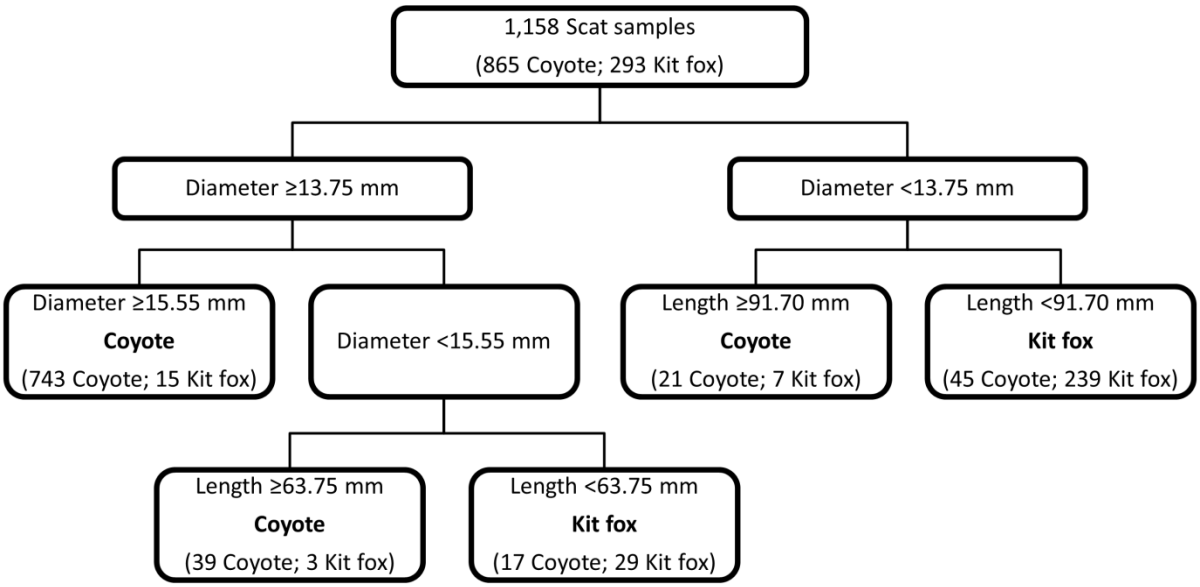



**Figure 11. Classification tree for coyote and kit fox scats collected in Tooele County, Utah (USA) in the winter and summer of 2013. Terminal nodes indicate the predicted class (bold) based on the decision rules leading to the node and the number of each species that was classified to the node (source: Lonsinger et al. 2015b).**

These results suggest that field identification of carnivore scats can suffer from high misclassification rates, even when sympatric species have disparate body sizes. Inaccurate species identification can bias inferences drawn from scat surveys and may lead to less effective management strategies. We encourage resource managers and researchers utilizing scat surveys to employ methods to minimize or eliminate misclassifications. While unambiguous molecular identification provides reliable classification, managers conducting long-term monitoring, surveys over large spatial extents, and/or working with limited funding may not be able to utilize molecular identification for the duration of a monitoring program or study. Alternatively, nonparametric classification based on morphometric characteristics may decrease misclassification rates over field identification. Approaches that elucidate areas of greatest misclassification, such as classification trees where misclassification rate can be identified by node, can be used to direct molecular identification analyses to those samples most likely to be misidentified, reducing overall misclassification while keeping costs low. Additionally, for studies employing molecular identification, classification techniques may provide an avenue for reliably identifying scats that fail molecular identification, due to DNA degradation; this may be particularly important in environments where fecal DNA degrades more rapidly.

**Table 4. Misclassification rates based on field identification (ID), *k*-nearest neighbor classification (KNN), and classification trees (CT) for carnivore scats collected in western Utah, USA, during the winter and summer of 2013. The misclassification rate was the proportion of samples classified to a species that was in disagreement with molecular identification as determined with mitochondrial DNA. Only scats for which measurements of diameter, length, and number of disjoint segments were available were evaluated. The lowest mean misclassification rates for KNN were achieved at *k* = 3 (overall and kit foxes) and *k* = 7 (coyotes; source: modified from Lonsinger et al. 2015b).**

| | Misclassification rate | | |
|---|---|---|---|
| Scat type | Field ID[a] | KNN[b] | CT[b] |
| Overall | 12.2% | 11.7% | 7.5% |
| Kit fox | 22.9% | 8.1% | 8.5% |
| Coyote | 7.1% | 12.4% | 7.2% |
| *n* = | 1,203 | 1,158 | 1,158 |

[a] Misclassification rate incorporates all carnivore scats identified to species with molecular identification.
[b] Misclassification rate incorporates only scats identified as kit fox or coyote with molecular identification.

*Kit fox scat removal*

For carnivores, such as kit foxes, scat surveys are typically conducted along linear features, such as roads and trails (Kohn et al. 1999, Schauster et al. 2002, Gompper et al. 2006, Schwalm et al. 2012, Dempsey et al. 2014). While decay, deterioration and biotic displacement may reduce the number of carnivore scats available for detection over extended time periods (Sanchez et al. 2004, Livingston et al. 2005), anthropogenic sources of removal along roadways (e.g., vehicles) or trails (e.g., foot-traffic, off-road vehicles) may operate more rapidly. Accelerated scat removal rates and/or inequitable removal rates among survey sites or target species, may bias results of scat surveys. We used experimental plots to evaluate variation in scat removal for two model carnivores, kit foxes and coyotes, along roads at DPG. Using parametric survival regression, we predicted scat survival and developed persistence-rate correction factors, which we applied to results from relative abundance scat surveys. Although this demonstration pertains only to kit foxes, our analysis for kit foxes and coyotes were completed concurrently. Consequently, we present the results for both kit foxes and coyotes here. Detailed results are published in Lonsinger et al. (2016).

We conducted scat removal experiments along gravel (maintained) and two-track dirt (unmaintained) roads during summer 2013 and winter 2014. We identified three common road types across our study site: (i) two-lane gravel (large), (ii) one-lane gravel (medium) and (iii) two-track (small) roads. We then established three removal plots on roads representing each strata. In each season, we placed 90 coyote and 90 kit fox scats from captive animals across nine

removal plots. We placed 10 scats of each species in each plot, with scats placed ~5 m apart and alternating between species, resulting in 30 scats per road type per species. Furthermore, we systematically positioned scats either on the median, tire tracks or shoulder, so that among the 90 scats per species, each position was represented by 30 scats evenly distributed across road types and plots. We initiated scat removal experiments on 29 July 2013 (summer) and 12 January 2014 (winter) and monitored removal of scats on each plot at 1, 3, 5, 7, 11, 14, 21, 28 and 42 days after setting, tracking the fate of each scat separately. We monitored vehicle traffic at each plot with traffic counters and calculated the mean daily vehicle rate for each plot each season. Although all scats were placed directly on transects, we estimated natural decay and disappearance rates by evaluating the proportion of scats that disappeared from plots during intervals when no traffic was recorded.

Overall, 3.3% of kit fox scats and 10.6% of coyote scats persisted through 42 days. When comparing overall scat persistence by road type, 13.3%, 6.7% and <1.0% of scats on small, medium and large roads, respectively, persisted through 42 days. At 14 days (a common sampling interval for relative abundance estimation), the proportion of scats removed was 90.8% for large roads, 64.2% for medium roads and 41.7% for small roads. By position, 10.0% and 10.8% of scats on the shoulder and median, respectively, persisted through 42 days, while no scats in tracks persisted to 42 days; 87.5% of scats in tracks were removed by day 14. We observed similar levels of overall persistence between seasons (proportion persisting to 42 days: summer = 7.2%; winter = 6.7%). Across road types, daily traffic rates were higher in summer (overall mean = 20.8 ± 10.6 SE) than winter (overall mean = 10.7 ± 5.5 SE). Across replicates, daily traffic rates were generally higher for large and medium roads than for small roads (Table 5). During periods with no traffic, persistence rates across seasons were high for kit fox (93.5%) and coyote (93.0%) scats. When considering the number of scats available and duration of intervals without traffic, natural removal for kit fox and coyote scats occurred at a rate of 0.11 and 0.12 scats/day per 100 scats, respectively.

**Table 5. Daily traffic volume (mean number of vehicles per day over 42 days) for nine experimental removal plots used to investigate coyote and kit fox scat removal in western Utah, during two seasons. Plots were distributed across large (two-lane gravel), medium (one-lane gravel), and small (two-track) roads. Overall mean ± SE for each road type is across seasons and replicates (source: Lonsinger et al. 2016).**

| | Small | | | Medium | | | Large | | |
|---|---|---|---|---|---|---|---|---|---|
| | Plot 1 | Plot 2 | Plot 3 | Plot 4 | Plot 5 | Plot 6 | Plot 7 | Plot 8 | Plot 9 |
| Summer 2013 | 1.29 | 0.17 | 1.00 | 25.21 | 0.74 | 2.43 | 6.69 | 65.54 | 83.68 |
| Winter 2014 | 0.48 | 0.19 | 0.12 | 11.33 | 0.98 | 1.69 | 4.07 | 33.57 | 43.57 |
| Mean ± SE | 0.54 ± 0.20 | | | 7.03 ± 3.98 | | | 39.52 ± 12.93 | | |

We employed accelerated failure time parametric (exponential) survival models with interval censoring to investigate the effects of species, season, position, road type and mean daily vehicle traffic on scat removal (Pyke and Thompson 1986, Hosmer et al. 2008). Species served as a surrogate for scat size (Lonsinger et al. 2015b). Season represented climatic differences between periods. Road type characterized road size and condition, which regulated vehicle speeds (intensity of disturbance). Traffic represented the mean daily vehicle passage rate (frequency of disturbance). We evaluated models with all possible combinations of main effects and a null model and evaluated model fit based on Akaike's Information Criterion with small sample size correction (AICc; Hurvich and Tsai 1989).

The top model included four predictor variables: species, road type, position and traffic (Table 6). The second model included these same predictors plus season and was 2 ΔAICc from the top model (Table 6), suggesting there was little support for this additional parameter or model (i.e., a pretending variable; Arnold 2010). The next closest model was >10 ΔAICc from the top model (Table 6). The Akaike weight indicated that given the candidate model set and data, the top model received 73% of the support and the cumulative Akaike weight of the top two models was >99%, providing a high level of support that the four variables common to both models were important predictors (Table 6).

**Table 6. Ranking of parametric survival regression models for carnivore scat removal base on Akaike's Information Criterion with small sample size correction (AICc). Explanatory variables included species, road type, scat position (shoulder, track and median), season, and mean daily traffic volume. Each model is ranked based on ΔAICc, where K = number of model parameters, $w_i$ = Akaike weight and LL = log-likelihood. Only the top four models and null model are presented (source: Lonsinger et al. 2016).**

| Model | K | $AIC_c$ | $\Delta AIC_c$ | $w_i$ | LL |
|---|---|---|---|---|---|
| Position + Road + Species + Traffic | 7 | 1386.74 | 0 | 0.730 | -686.2 |
| Position + Road + Species + Traffic + Season | 8 | 1388.76 | 2.03 | 0.265 | -686.2 |
| Position + Road + Traffic | 6 | 1397.44 | 10.70 | 0.003 | -692.6 |
| Position + Road + Traffic + Season | 7 | 1399.51 | 12.78 | 0.001 | -692.6 |
| Null | 1 | 1686.49 | 299.76 | 0.000 | -842.2 |

Larger coyote scats survived longer than the smaller kit fox scats (Table 7). Scats deposited in the median survived longer than those in the tracks; scats on the shoulder persisted the longest (Table 7). Scats on medium and small roads had 1.6 and 3.5 times longer survival, respectively, than scats on large roads (Table 7). Vehicle traffic was negatively associated with scat survival (Table 7).

The proportion of scats persisting through a discrete time period can be used to determine a persistence-rate correction factor (Brodie 2006). We used the top model to predict scat survival for each combination of species, road type and position over time based on the exponential

survival function (Hosmer et al. 2008). While we were able to obtain mean daily traffic volumes for each of our removal plots, in practice, estimates of traffic are rarely available for each scat transect. More realistically, traffic estimates may be available for a small number of roads, which are representative of road types surveyed. For each road type, we calculated an overall mean daily traffic volume by combining mean daily traffic estimates across replicates and seasons. We then used the overall mean daily traffic volume for each road type when predicting scat survival over time.

**Table 7. Regression coefficients, standard errors (SE), and *p*-values of the best fitting exponential survival model for carnivore scat persistence assessed by Akaike's Information Criterion with small sample size correction. Species included coyote and kit fox, road type included large (two-lane gravel), medium (one-lane gravel) and small (two-track) roads and position included the median, track, and shoulder. Traffic accounts for the mean daily number of vehicles passing sites (source: Lonsinger et al. 2016).**

| Parameter | Coefficient | SE | *P*-value |
|---|---|---|---|
| Intercept | 2.3652 | 0.192 | <0.001 |
| Shoulder (Position) | 0.4187 | 0.142 | 0.003 |
| Track (Position) | -1.2013 | 0.139 | <0.001 |
| Medium (Road type) | 0.4733 | 0.176 | 0.007 |
| Small (Road type) | 1.2418 | 0.186 | <0.001 |
| Kit fox (Species) | -0.4046 | 0.113 | <0.001 |
| Traffic | -0.0192 | 0.003 | <0.001 |

For prediction, we applied parameter estimates from the top model to the exponential survival function. The exponential survival function describing survival ($S$) over time (t) is $S(t) = \exp(-\lambda t)$, where $\lambda = \exp(-\beta o - \beta 1 x1 - \ldots - \beta i\ xi)$; $\beta o$ and $\beta i$ represent the regression parameters for the intercept and predictor variable i, respectively, while xi represents the value of predictor variable i under consideration (Hosmer et al. 2008). Based on this survival function and the parameter estimates from the top model, we estimated the proportion of scats surviving for 1–42 days for all possible combinations of species, road type and position, and applying the overall mean daily traffic for each road type. The resulting proportions constituted our persistence-rate correction factors (Brodie 2006). To further explore the role of traffic, we evaluated the decimating effect of traffic by predicting mean time until scat removal for each combination of predictor variables and considering mean daily traffic values from 1–84 (the highest observed traffic).

Survival decreased over time for coyote and kit fox scats, with survival declining more precipitously along larger roads and for scats positioned in the tracks and median (Figure 12). Scats were unlikely to persist through 42 days when deposited on the tracks, regardless of road type or species. As mean daily traffic increased, survival time decreased (Figure 13). Predicted

time until removal was low for scats deposited on tracks, even with low traffic levels. Coyote scats were predicted to persist longer than kit fox scats (Figure 13).

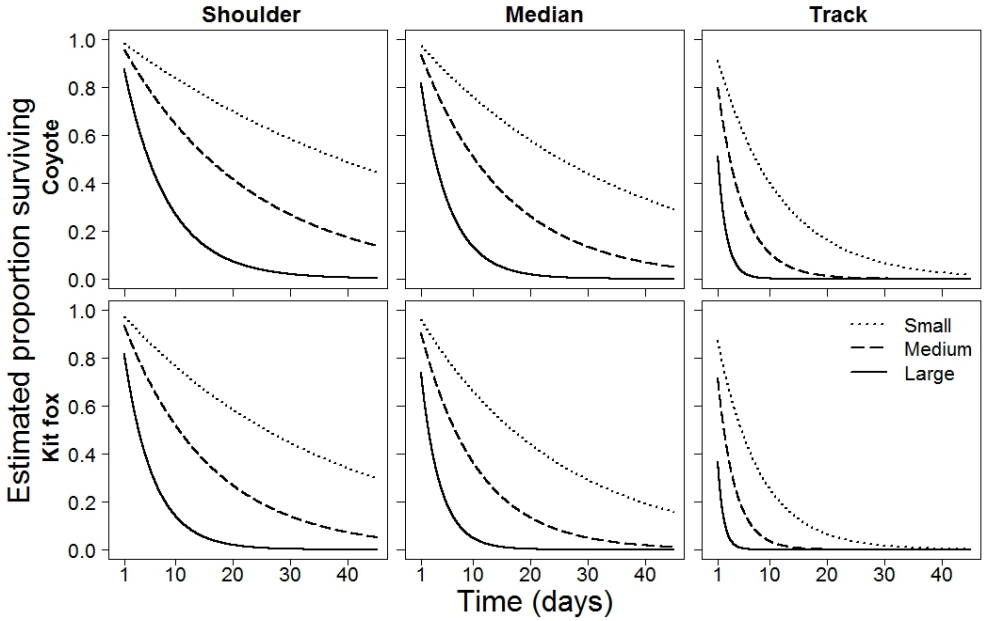



**Figure 12. Estimated proportion of coyote and kit fox scats surviving over time on large (two-lane gravel), medium (one-lane gravel) and small (two-track) roadways when deposited in the median, track, or shoulder in western Utah, USA. Estimated survival was based on the exponential survival function assuming a mean daily traffic volume for each road type (large = 39.5; medium = 7.03; small = 0.54; source: Lonsinger et al. 2016).**

To evaluate the influence that removal has on inferences from relative abundance surveys, we cleared and subsequently surveyed 15 transects (5 km each) three (summer 2013) to four (winter 2014) times for all carnivore scats (Schauster et al. 2002, Dempsey et al. 2014). Transects followed roads with characteristics similar to those used for scat removal experiments. For each carnivore scat detected, we (1) collected ~0.7 mL of fecal material into 1.4 mL of DETs buffer (Seutin et al. 1991), (2) measured the diameter, length, and number of segments (Lonsinger et al. 2015b), and (3) recorded the location and position (median, track or shoulder), before (4) removing remaining portions. We identified scats to species using mtDNA (De Barba et al. 2014) and a site-specific non-parametric classification tree with high accuracy based on measurements (Lonsinger et al. 2015b).

We collected 554 (summer: n = 363; winter: n = 191) carnivore scats. Which were as identified as originating from coyotes (361), kit foxes (170), bobcats (18), red foxes (4), and unknown (1). We excluded bobcat, red fox, and the one unknown scat from subsequent analyses. We calculated the relative abundance of coyotes and kit foxes for each transect in each season as the mean number of scats detected across temporal replicates.

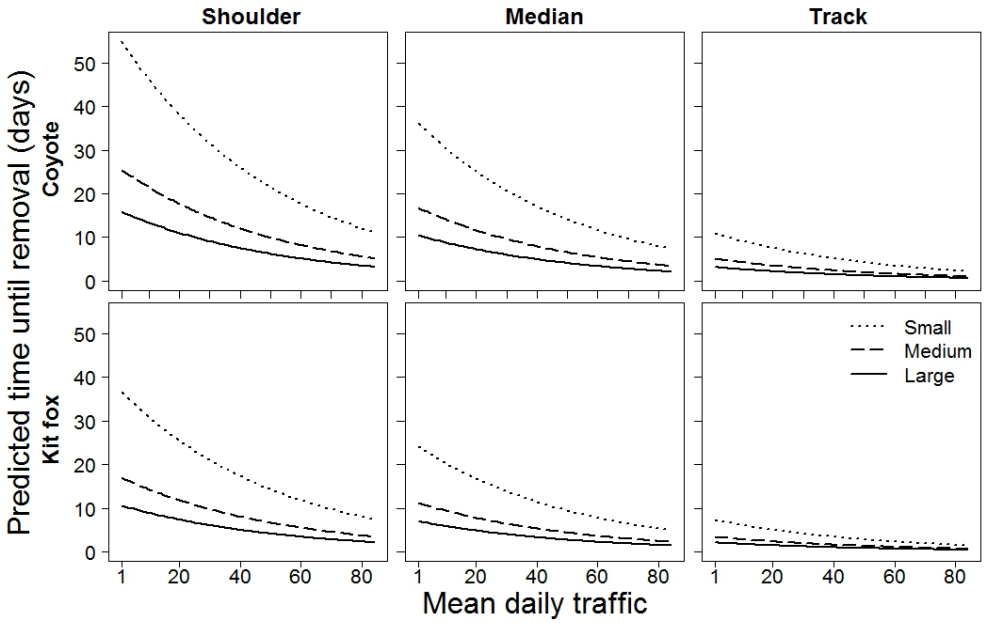



**Figure 13. Predicted time until removal for coyote and kit fox scats as a function of mean daily vehicle traffic on large (two-lane gravel), medium (one-lane gravel) and small (two-track) roadways when deposited in the median, track or shoulder in western Utah, USA (source: Lonsinger et al. 2016).**

For each species-season combination, we ranked and compared the relative abundance of transects. To correct for removal, we then categorized each transect by road type and each scat by position. For each temporal survey of each transect, we used the survival function resulting from the top exponential regression model to develop a persistence-rate correction factor for each combination of species, road type and position. For each transect in each season, we identified the removal plot of the same road type that best reflected the amount of traffic on the transect, and used the corresponding mean daily traffic when developing the transect and survey specific persistence-rate correction factors. For each species, we then calculated the corrected relative abundance by dividing the number of scats detected on each road type and in each position during a survey, by the transect and survey specific persistence-rate correction factor; within a survey, these values were then summed to obtain the corrected survey-specific number of scats per species. We then re-evaluated the rank and relative abundance of transects for each species-season combination based on corrected relative abundance, and compared this to the uncorrected relative abundance by calculating the ratio of corrected to uncorrected relative abundance.

Six transects contained two road types (Lonsinger et al. 2016) and therefore six correction factors were applied to each species in each season. The remaining nine contained a single road type and three correction factors were applied to each species-season combination. We detected coyotes across 15 (summer) and 13 (winter) transects, and kit foxes across 14 (summer) and 9 (winter) transects (Table 8). Correcting relative abundance for inequitable removal altered the rankings of transects for both species in both seasons (Table 8). Corrected:uncorrected relative abundance ratios were generally higher in summer than winter for both species (Table 8). In both

seasons, corrected:uncorrected relative abundance ratios were highest on those transects characterized as large roads with the highest traffic volumes (transects 4, 5 and 15), when the target species was detected (Table 8).

**Table 8. Relative abundance (RA), corrected relative abundance (cRA), and ratio (R; cRA/RA) for coyotes and kit foxes along 15 transects (Tran) in western Utah, USA, over two seasons. Corrected relative abundance incorporates a persistence-rate correction factor estimated by scat removal experiments (source: Lonsinger et al. 2016).**

| | Coyote | | | | | | Kit Fox | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Summer 2013 | | | Winter 2014 | | | Summer 2013 | | | Winter 2014 | | |
| Tran | RA | cRA | R | RA | cRA | R | RA | cRA | R | RA | cRA | R |
| 1 | 5.7 | 24.6 | 4.3 | 2.8 | 27.3 | 9.8 | 0.7 | 1.7 | 2.4 | 0.0 | 0.0 | |
| 2 | 4.3 | 19.7 | 4.6 | 1.0 | 1.9 | 1.9 | 2.0 | 4.7 | 2.4 | 0.0 | 0.0 | |
| 3 | 6.3 | 16.9 | 2.7 | 1.3 | 2.2 | 1.7 | 1.0 | 3.5 | 3.5 | 2.8 | 12.0 | 4.3 |
| 4 | 1.3 | 118.6 | 91.2 | 0.0 | 0.0 | | 0.3 | 333.4 | 1111.3 | 0.0 | 0.0 | |
| 5 | 1.7 | 61.6 | 36.2 | 0.0 | 0.0 | | 1.0 | 95.0 | 95.0 | 0.0 | 0.0 | |
| 6 | 7.0 | 17.1 | 2.4 | 0.3 | 0.3 | 1.0 | 0.3 | 2.3 | 7.7 | 0.3 | 1.7 | 5.7 |
| 7 | 10.7 | 27.4 | 2.6 | 5.3 | 12.2 | 2.3 | 1.3 | 3.7 | 2.8 | 1.3 | 5.7 | 4.4 |
| 8 | 7.0 | 12.5 | 1.8 | 4.3 | 9.6 | 2.2 | 3.0 | 9.8 | 3.3 | 0.0 | 0.0 | |
| 9 | 3.0 | 5.6 | 1.9 | 1.8 | 3.5 | 1.9 | 17.0 | 45.7 | 2.7 | 8.8 | 29.1 | 3.3 |
| 10 | 1.3 | 7.2 | 5.5 | 0.3 | 0.4 | 1.3 | 4.3 | 34.5 | 8.0 | 1.0 | 1.7 | 1.7 |
| 11 | 5.0 | 11.7 | 2.3 | 5.3 | 40.8 | 7.7 | 0.0 | 0.0 | | 0.8 | 3.7 | 4.6 |
| 12 | 5.0 | 9.0 | 1.8 | 1.8 | 7.0 | 3.9 | 0.3 | 0.5 | 1.7 | 0.3 | 0.4 | 1.3 |
| 13 | 14.3 | 34.5 | 2.4 | 4.3 | 9.9 | 2.3 | 0.7 | 1.1 | 1.6 | 0.0 | 0.0 | |
| 14 | 6.3 | 159.1 | 25.3 | 0.8 | 29.5 | 36.9 | 2.0 | 12.0 | 6.0 | 1.5 | 5.0 | 3.3 |
| 15 | 2.7 | 106.6 | 39.5 | 0.3 | 4.2 | 14.0 | 0.3 | 210.4 | 701.3 | 0.3 | 16.9 | 56.3 |

Monitoring programs employing scat surveys are often interested in evaluating relative abundance (Gese 2001), occupancy patterns (Long et al. 2011) or demographic parameters (Lukacs and Burnham 2005). Our results suggest that failure to account for spatial variation in scat removal may bias results of monitoring programs, leading to erroneous conclusions and/or ineffective management decisions. Disparity in scat removal among species stresses the importance of understanding interspecific variation in removal rates, particularly when employing multi-species monitoring. The effects of road type and position have important

implications for study design and analyses. Larger roads may yield fewer scats and are more likely to produce low detection probabilities and false-negatives (Rhodes et al. 2011); it may be advantageous to survey smaller roads or trails in lieu of larger roads, whenever possible. When using scat surveys to conduct occupancy or capture-recapture modeling, incorporation of road type as a site level covariate may effectively account for some detection or capture heterogeneity and improve model fit (Lukacs and Burnham 2005). Understanding spatial variation in removal by position and road type allows researchers to conduct informed subsampling to reduce the probability of false-positives (Rhodes et al. 2011). If road type and position are documented during surveys, persistence-rate correction factors can adjust for variation in removal among road types and positions. We caution though, that when correcting relative abundance for removal, transects experiencing high removal rates, such as those observed on large roads in our system, are likely to introduce greater bias and produce very high corrected:uncorrected relative abundance ratios. Given the potential variation in bias introduced by disparity in removal rates, we encourage practitioners employing scat surveys along roads or trails to explicitly consider the potential implications of removal by anthropogenic impacts.

*Pronghorn degradation and deposition*

These results are published in Woodruff et al. 2014 and Woodruff et al. 2015.

To ensure collection of samples less than 24 hours old, the area around feed stations in the captive pen on CPNWR was cleared of pellets on 3 July 2012. On 4 July 2012, we returned and collected 20 pellet piles from ten presumed adult (five males, five females) and 10 presumed fawn (five males, four females, one unknown sex). Fawn and adult samples were classified based on visual inspection of size and morphology (thus, "presumed"). Our manuscript is published in Wildlife Biology (Woodruff et al. 2016a) documenting size differences in fecal pellets of fawn and adult pronghorn. Differences have been documented for other ungulates (Ezcurra and Gallina 1981, Bubenik 1982, MacCracken and Van Ballenberge 1987, Sanchez-Rojas et al. 2004). Pellet piles were exposed to local environmental conditions near the captive pen for 124 days from 4 July 2012 through 5 November 2012. Three pellets were collected from each sample at days 1, 3, 5, 7, 14, 21, 60, and 124, placed in paper coin envelopes, and stored at room temperature in a plastic Ziploc bag with one cup of silica desiccant (Fisher catalogue no. S161-212) to reduce DNA degradation prior to analysis. We tracked rainfall and temperature during the sampling period using local weather stations (http://www.earthonly.com/ajo/weather/). Average high temperatures were ~39 ˚C from day 1 to 60 and 33 ˚C from days 60 to 124, and total rainfall from day 1 to 124 was 16.4 cm. We evaluated the rate of mtDNA and nDNA degradation in these samples ranging from 1–124 days old and documented that mtDNA species identification success rates were 100% through day 14. Success rates dropped to 95% by day 21, 50% on day 60, and 10% by day 124 (Figure 14). Average amplification success rates for six nDNA microsatellite loci were 81% for samples on day one, 63% by day seven, 2% by day 14, and 0% by day 60 (Figure 15).

As part of our pilot study, we designed a species identification test using mitochondrial DNA (mtDNA) species-specific primers to distinguish between Sonoran pronghorn and mule deer (*Odocoileus* hemionus) using DNA extracted from fecal pellets (Woodruff et al. 2014). Pronghorn are the primary ungulate species present at most drinkers. However, mule deer can

visit the sites and similarities between pronghorn and deer pellets make it impossible to distinguish by visual inspection (Johnson and MacCracken 1978). We accurately identified each species in 100% of blood and tissue reference samples. Mule deer samples do not amplify at our selected microsatellite loci, so we performed mtDNA species ID only on failed samples to discriminate between pronghorn and mule deer samples and calculated individual ID success rates for pronghorn samples only. Nine and 29 samples were mule deer in 2013 and 2014, respectively.

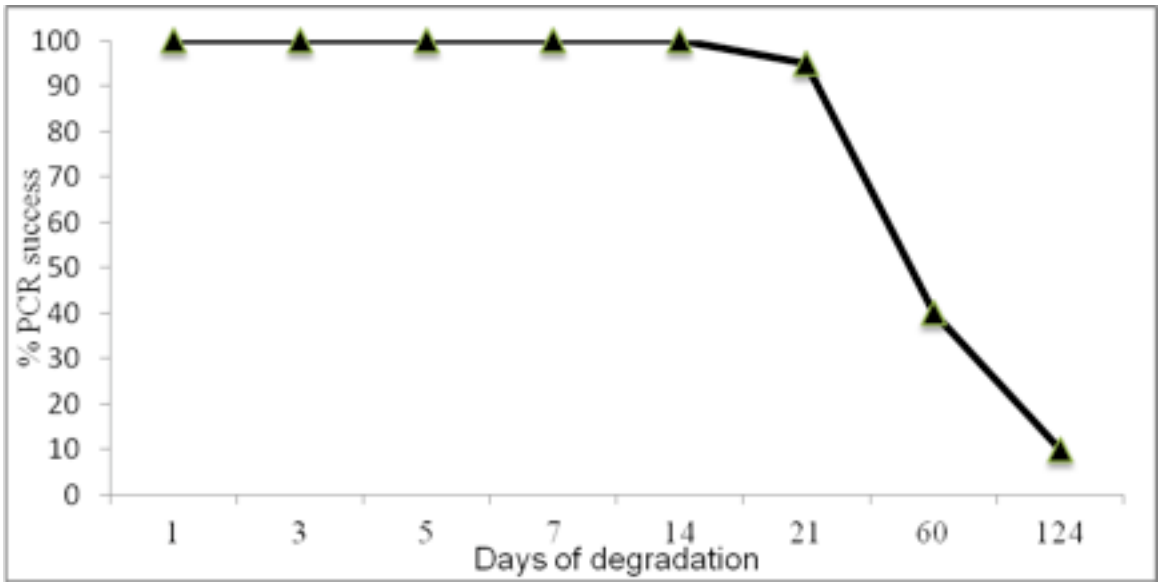



**Figure 14. Percent PCR success from day 1–124 for mtDNA for Sonoran pronghorn fecal pellets.**

To evaluate optimal sampling intervals for mark-recapture analysis, deposition data was also collected at five drinkers. In July 2012, we collected deposition data for Sonoran pronghorn at 2 sites in CPNRW, and 3 sites in BMGR. First, we cleared a 25 meter radius around the drinker and feeding area sites of all fecal pellets then waited 1–7 days and counted fecal pellets. Deposition averaged one pellet pile per pronghorn per day (range: 4 to 43; Table 9). Deposition rates varied depending on the number of days of between observations, number of animals known to use the site, and the local weather conditions. Fecal pellet deposition decreased after rain events, when pronghorn presumably found water from natural sources, highlighting the importance of focusing our sampling in the dry season.

We also evaluated sampling interval efficiency for designing protocol for sample collection for Year 2 and Year 3 mark-recapture analysis. We assumed 15 pronghorn per site (range: 6–25 per site), an estimated deposition rate of one pellet pile per pronghorn per day, and an average sample removal rate of 10% per day. We acknowledge that removal rates vary by drinker; however, we used this average value of removal rates at East Release (Table 9) for modeling purposes. Thus, the number of samples available for collection is a product of the number of pronghorn using the site and the number of days in the interval, minus 10% per day. For example, with 15 pronghorn and a sampling interval of four days, 15 samples are 1 day old, 14 are 2 days old, 13 are 3 days old, 12 are 4 days old, and so on. We then used our model-based predicted PCR success rates to

estimate the number and percent of samples that could be successfully genotyped at each sampling interval from 1 to 10 days. At every sampling interval, each sample was assigned a specific predicted PCR success dependent on sample age.

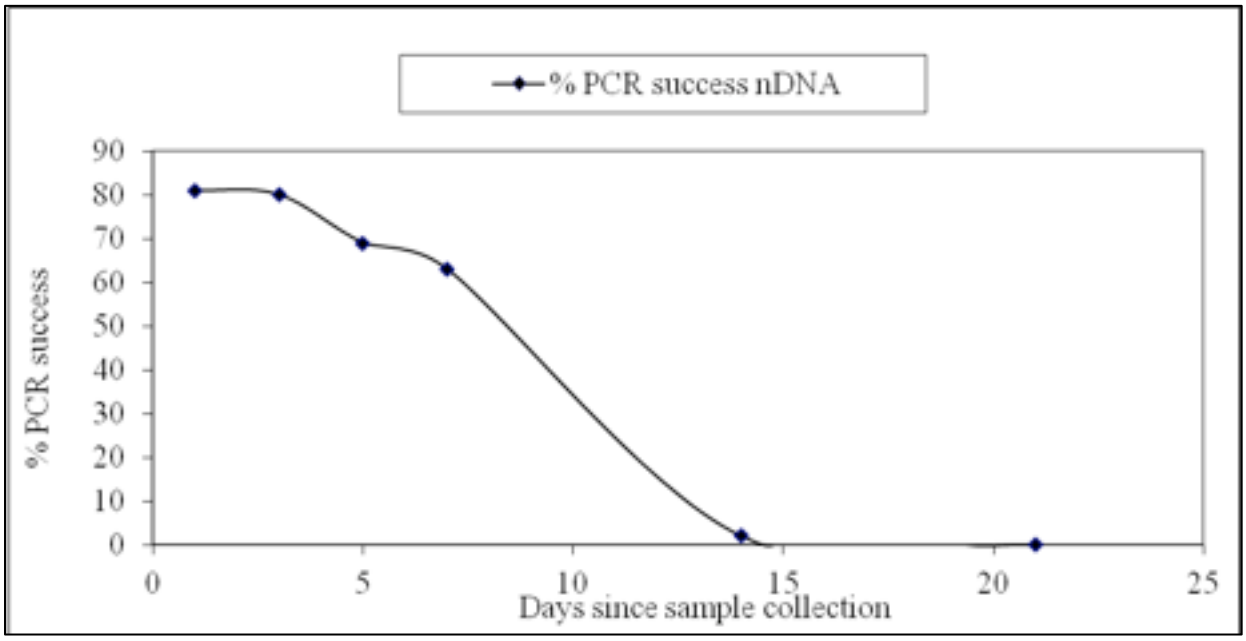



**Figure 15. Percent PCR success from day 1–21 for nDNA for Sonoran pronghorn fecal pellets. PCR success rates were 0 for longer than 21 days.**

**Table 9. Deposition rates for Sonoran Pronghorn fecal pellets collected in Cabeza Prieta National Wildlife Refuge and Barry M. Goldwater Range, Arizona, USA during July 2012.**

| Drinker | Sampling Interval (days) | # Pellet Piles | Average Pellet Piles/Day | Est. # pronghorn present | Average # piles/pronghorn/ day |
|---|---|---|---|---|---|
| East Release | 1 | 9 | 9 | 7 | 1.29 |
| Charlie Bell | 1 | 7 | 7 | 9 | 0.78 |
| East Release | 2 | 19 | 10 | 8 | 1.19 |
| Uken | 3 | 129 | 43 | 25 | 1.72 |
| East Release | 6 | 37 | 6 | 8 | 0.77 |
| Uken | 7 | 135 | 19 | 25 | 0.77 |
| Point of Pintas | 7 | 31 | 4 | 7 | 0.63* |
| Devil Hills | 7 | 30 | 4 | 11 | 0.39* |

***outliers are due to rain events**

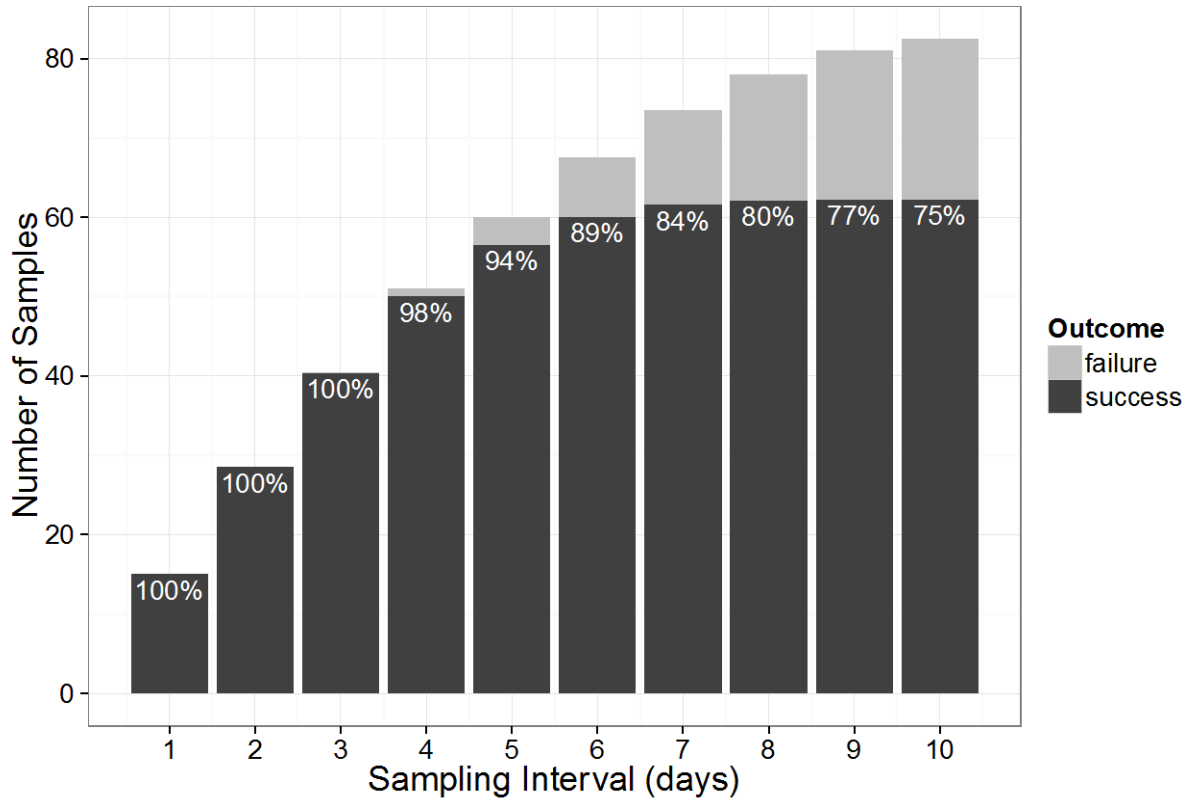



**Figure 16. Expected number of failed and successful Sonoran pronghorn fecal pellets samples by sampling interval (days). The percentages are the relative efficiency (i.e., percent of expected successful samples).**

Based on individual ID success, a sampling interval of 1–7 days (Figure 16) would be sufficient to optimize amplification success rates; however, an interval of 1–3 days would likely give too small a sample size, and local managers attempt to limit disturbance of pronghorn at drinkers to once per week. Sampling every 4–5 days is the ideal balance between DNA degradation and deposition. However, in order to minimize disturbance we proposed using a 7 day sampling interval and synchronizing weekly agency personnel visits for stocking feed and water with fecal DNA sample collection.

*Determining age class for Sonoran pronghorn*

Assigning age to an individual to track it throughout its lifetime usually involves capture and handling. Consequently, the use of noninvasively obtained DNA samples is advantageous. One weakness of this method, however, is the difficulty of aging individuals with noninvasive genetic samples, yet understanding the age structure of a population is central to understanding age-specific survival and recruitment. We measured Sonoran pronghorn fecal pellets (length, width, length-width ratio, volume) collected post-fawning and matched to known age captive animals using fecal DNA genotyping to determine the feasibility of distinguishing age class by pellet dimensions.

Annual capture operations are conducted in the captive pen by Arizona Game and Fish Department (AZGFD) and US Fish and Wildlife Service (USFWS), during which individuals are captured, radio-collared, and a blood sample is collected. Fawns in the pen are tracked from birth when possible and captured fawns are ear-tagged, and radio-collared if re-captured in subsequent captures. Young of the year are easily identified (e.g., size, horn development) and classified as fawn (0–11 months), and individuals captured as fawns in the previous year are known yearlings (12–23 months). However, not all fawns are caught during capture operations and consequently, an individual may not be handled until it is >1 year old and is potentially misclassified as to actual age. Thus, all captured animals of unknown age are classified as adults. Some captured (captive) individuals are subsequently released into the wild, at a ratio of approximately two males to one female (UWFWS 2015). To obtain DNA, blood samples (hereafter reference samples) were collected from 58 captured individuals in December 2012 and 2013 when feasible (i.e., if health and safety of the animal was not at risk due to stress) (USFWS 2015). These samples provided a genotype of an individual of known age for later matching to genotypes obtained from fecal pellets collected in the pen.

In May 2012 in the captive pen, we collected five fecal pellets (Morden et al. 2011) less than 24 hours old from each of 185 fecal pellet piles in three pellet size groups defined visually as small, medium, and large (size of pellet, not size of pile). While we recognize our size classification is subjective, we wanted to ensure collection of all age and sex classes, and this size classification was used only to structure collection and was not part of the analyses. To determine the age of the individual from which the fecal sample was collected, we matched 7–16 locus microsatellite genotypes of fecal samples to the reference samples.

Based on cross-validation with logistic regression predictive models, we estimated a 98% probability of correct classification of fawn versus yearling and fawn versus adult using pellet width as a single explanatory variable (Woodruff 2015, Woodruff et al. 2016a). We could not, however, distinguish between yearling and adult. We additionally evaluated our ability to classify age class of fecal pellets by visual assessment only, and this approach was unreliable. Thus, we recommend measuring pellets for more accurate age classification. This measurement method is simple, inexpensive, and shows potential for use in wild populations of pronghorn to discriminate fawns from other age classes. When combined with individual identification using fecal DNA, this method could provide better knowledge of recruitment and age-specific survival.

## 2.3 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

*Noninvasive Genetic Sampling Capture-Recapture (NGS-CR)*

When compared to traditional techniques, NGS-CR has the potential to provide reliable estimates while requiring fewer resources and reducing stress to animals (Waits and Paetkau 2005, Luikart et al. 2010). Another benefit of NGS-CR is that the genetic data can also be used to estimate other important indicators of population health including genetic diversity, population connectivity and effective population size ($N_e$), a critical population genetic parameter related to inbreeding and population viability (Schwartz et al. 2007, Luikart et al. 2010).

One challenge of NGS-CR is a concern about poor data quality caused by DNA degradation and genotyping errors, which can be common with noninvasive DNA samples (Taberlet et al. 1999, Waits and Paetkau 2005). Considerable effort has been expended to understand how sample age (Piggott 2004, Murphy et al. 2007, Santini et al. 2007), environmental conditions (Piggott 2004, Murphy et al. 2007, Santini et al. 2007, DeMay et al. 2013), diet (Murphy et al. 2003, Panasci et al. 2011), sample collection and storage techniques (Murphy et al. 2002, Palomares et al. 2002; Piggott and Taylor 2003, Stenglein et al. 2010a, Panasci et al. 2011), locus length (Buchan et al. 2005, DeMay et al. 2013, Lonsinger et al. 2015a) and species-specific differences (Piggott and Taylor 2003, Buchan et al. 2005, Lonsinger et al. 2015a) influence DNA degradation. These studies indicate DNA degradation and genotyping errors vary among species and environmental conditions. Field recommendations to reduce degradation and genotyping errors include sampling the freshest scats and conducting surveys during the driest and/or coldest seasons (Murphy et al. 2007, Santini et al. 2007).

Fortunately, new laboratory techniques and estimation models continually improve our ability to limit effects of genotyping errors (e.g. Wright et al. 2009, Beja-Pereira et al. 2009). Laboratory and analysis procedures aimed at minimizing genotyping errors include (i) using of a multi-tubes approach (Taberlet et al. 1996), (ii) culling of low quality samples failing mtDNA species identification (Kohn et al. 1999), (iii) dropping low quality samples that fail to amplify at >50% of nDNA loci in 2 initial replicates (Paetkau 2003), (iv) requiring that alleles of heterozygous and homozygous genotypes be observed ≥2 and ≥3 times, respectively (Frantz et al. 2003), (v) requiring consensus genotypes across a sufficient number of loci to ensure a probability that two siblings have identical multilocus genotypes (P(ID)sibs) < 0.01 (Waits et al. 2001), (vi) removing samples that failed to achieve consensus genotypes at a sufficient number of loci, (vii) comparing samples with identical or near identical multilocus genotypes and re-evaluating scoring of near matches to check for inconsistencies (Creel et al. 2003), (viii), considering consistency in sex identification and comparing inter-sample distances between matches (Smith et al. 2006), and (ix) evaluating the reliability of multilocus genotypes observed only once with the program RELIOTYPE (Miller et al. 2002). In this demonstration we implemented all of these precautions. Additionally, as part of this demonstration we developed an R based script, ConGenR, which facilitates the rapid determination of consensus genotypes from replicated samples, determines overall and individual sample level amplification success rates, and quantifies genotyping error rates (Lonsinger and Waits 2015). ConGenR is intended for use with samples collected noninvasively and processed with a multi-tubes approach. ConGenR can evaluate samples by class (i.e., any identifiable and meaningful subdivision of samples; e.g., sex, season, region, or sample condition), offering insights into processes driving amplification success and genotyping error rates. Additionally, amplification success and genotyping error rates are calculated by locus, expediting the identification of problematic loci during pilot studies (Lonsinger and Waits 2015, R Core Team 2015).

Reliable parameter estimates using NGS-CR generally require sufficient sample sizes and capture probabilities and low capture biases (by sex, age etc.). Our pilot study in Year 1 was designed to estimate genotyping success and error rates, sample sizes, and capture probabilities so we could determine the optimal sampling design for Years 2 and 3 using simulation (e.g., Boulanger et al. 2004, Settlage et al. 2008, Robinson et al. 2009). Also, success rates were

maximized by using short (<200 base pair) microsatellite loci that were chosen from a test panel of ~20 loci.

*Kit Fox*

Both NGS-CR and NGS-OM approaches for kit fox may be influenced by weather conditions. During our initial sampling season (winter 2013), the study region experienced atypically high amounts of snowfall. While the presence of snow may act to preserve samples (i.e., samples collected off of snow tend to have higher amplification success rates), frequent snowfall may cover scats, making them unavailable to detection until the snow melts. Furthermore, this delayed availability may influence closer assumptions, particularly if scats are detected a long time after deposition and are still of high enough quality to amplify. Collectively, frequent snowfall can act to suppress capture/detection rates initially, and subsequently inflate capture/detection rates.

At DPG, NGS was conducted along roadways, a common approach used to monitor carnivores (Gese 2001, Dempsey et al. 2014). While this approach has the added benefit of increasing detection probabilities over alternative monitoring strategies (Schauster et al. 2002, Dempsey et al. 2014), it is restricted to sites with sufficient coverage of roads and or trails. At sites with limited or no road coverage, alternative sampling methodology would likely need to be considered; scat detection dogs have been successfully used to detect kit fox scats and offer an alternative approach in the absence of roadways (Smith et al. 2003). Still when sufficient road coverage exists, surveys along roadways may be easier to implement and require substantially less training and expertise for technicians.

One challenge of working along roadways is that of vehicle disturbance and scat removal. As is evidenced by the results of our scat removal experiments, scat removal varies both spatially and temporally, with vehicle traffic significantly influencing the persistence of scats and the results of surveys conducted along roadways (Lonsinger et al. 2016). Our results suggest that removal can vary significantly among roadways and that scats are unlikely to persist on roads with even low to moderate levels of traffic. Practitioners can minimize the influence of removal by avoiding survey routes that follow large or medium gravel roads (i.e., those roads with the highest traffic volumes and traffic speeds) and instead, conducting surveys along two-track roadways. Additionally, the influence of disturbance can be minimized by conducting surveys during periods with less vehicle traffic/activity. At our study site, traffic increases in the summer both on DPG (military training and exercises) and on neighboring federal lands (outdoor recreation).

*Noninvasive Genetic Sampling Occupancy Modeling (NGS-OM)*

Coupling NGS with occupancy modeling offers an efficient framework to investigate the spatial distribution and dynamics of species (MacKenzie et al. 2006). Employing NGS-OM may be favorable to NGS-CR in some situations. In particular, NGS-OM requires only species identification of scats, making NGS-OM a more cost-effective monitoring strategy than NGS-CR. Still, NGS-OM may fail to detect important population level changes in abundance, particularly with territorial species which may experience significant populations declines in abundance with little (or no) change in the proportion of area occupied (i.e., occupancy). Thus,

the decision to employ NGS-OM and/or NGS-CR techniques should depend on the parameters of interest to managers (e.g., abundance vs. occupancy, survival vs. local colonization and extinction). Kit fox are territorial, tend to live in pairs or small family groups, and have a high movement capacity. This, combined with the results of our mtDNA degradation experiment (i.e., samples as old as 4 months may produce successful species identification), suggests that occupancy may not provide the resolution to sufficiently detect changes in kit fox abundance. Still, occupancy offers insights into the processes driving space-use and can provide insights into the potential impact that military activities and trainings have on local extinction and/or colonization of plots. Because occupancy estimation requires only species identification, occupancy can be effectively implemented within a NGS-CR (which requires both species identification and individual identification), so long as the spatio-temporal sampling design accommodates both approaches, as we have demonstrated.

Parameter estimation within an occupancy modeling framework assumes no misidentification of species; misidentification can severely bias results (MacKenzie et al. 2006). Thus, we would caution against utilizing field based scat identification for evaluating kit fox occupancy patterns. Our results suggest that field identification of carnivore scats can suffer from high misclassification rates, even when sympatric species have disparate body sizes (Davison et al. 2002, Reed et al. 2004, Gompper et al. 2006, McCarthy et al. 2008, Harrington et al. 2010, Lonsinger et al. 2015b). Furthermore, misclassification were asymmetrical, with those species that are encountered less frequently being more often identified (incorrectly) as a more frequently detected species (Lonsinger et al. 2015b). We recommend that noninvasive monitoring programs incorporate a genetic species identification test to minimize or eliminate misidentification error. Although unambiguous molecular identification provides reliable classification, managers conducting long-term monitoring, surveys over large spatial extents, and/or working with limited funding may not be able to utilize molecular identification for the duration of a monitoring program or study. Alternatively, our results suggest that nonparametric classification based on morphometric characteristics may decrease misclassification rates over field identification. Approaches that elucidate areas of greatest misclassification, such as classification trees where misclassification rate can be identified by node, can be used to direct molecular identification analyses to those samples most likely to be misidentified, reducing overall misclassification while keeping costs low.

*Pronghorn*

Weather and forage conditions likely play a significant role in detection probabilities as drinker visitation declines in cooler, wetter conditions when there is adequate natural forage. During cooler, wetter times of year pronghorn are also spread out over a large spatial area (~11,000 $km^2$), and thus, sampling should be completed prior to the onset of monsoon season in July. We suspect when we initiated sampling in 2013 (see Results), drinker use was not at the maximum. Our inconsistent detection probabilities across sessions also provide explanation for why the best models included time variation (see Results).

Due to our targeted sampling design, any inference from our estimates applies largely to the individuals using drinkers. Twice as many males have been released from the captive pen potentially leading to a male bias using drinkers, as released animals, conditioned to being

provided supplemental feed and water, may use drinkers more readily (USFWS 2015). Additionally, home range size and movement rates likely differ between sexes (Ockenfels et al. 1994, Clemente et al. 1995) which could affect the use, and representation, of sexes at drinkers. These results suggest that our sampling method was better at sampling the male fraction of the population.

It should be noted that the timing of sample collection might vary from year to year with changing weather conditions. Lower drinker visitation will also result in lower detection probabilities and these differences need to be considered when monitoring population trends. Additionally, in the future, any extrapolation to the entire population, especially pertaining to male:female ratios, should include a sex ratio correction factor.

# 3.0 PERFORMANCE OBJECTIVES

**Table 10. Performance Objectives**

| Performance Objective | Metric | Data Requirements | Success Criteria | Results |
|---|---|---|---|---|
| **Quantitative Performance Objectives** | | | | |
| 1. Improved monitoring protocol for kit fox and Sonoran pronghorn based on NGS-CR | • Increase in the number of demographic parameters reliably estimated versus alternatives | • Number of demographic parameters that can be reliably estimated via NGS-CR | • Number of parameters obtained via NGS-CR is > number obtained from current approaches | • Kit fox: Yes<br>• Pronghorn: Yes |
| 2. Obtain reliable estimates of demographic parameters via implementation of NGS-CR monitoring protocol (kit fox and Sonoran pronghorn) | • Measures of precision for estimates of<br>1. Abundance<br>2. Survival<br>3. Reproduction<br>4. Population connectivity<br>5. Genetic diversity | • Mean values of parameter estimates<br>• Estimates of precision (e.g., standard error) for parameter estimates | • "Reliable" estimates for abundance are those with a coefficient of variation <10%<br>• For the other parameters, we evaluated only if it was possible to obtain the estimate from the available data | <u>Abundance</u><br>• Kit fox: Yes<br>• Pronghorn: Yes<br><u>Survival</u><br>• Kit fox: Yes<br>• Pronghorn: Yes<br><u>Reproduction</u><br>• Kit fox: No<br>• Pronghorn: Yes<br><u>Connectivity</u><br>• Kit fox: Yes<br>• Pronghorn: Yes<br><u>Genetic Diversity</u><br>• Kit fox: Yes<br>• Pronghorn: Yes |
| 3. Improve efficiency of current monitoring programs | • Increased cost-benefit of monitoring programs<br>• Increase in the spatial extent of area monitored versus alternatives | • Sampling design (i.e., area sampled, frequency and quantity of scat collection)<br>• Spatial area of inference for monitoring program | • The cost of obtaining each parameter based on NGS-CR is < cost of alternatives<br>• The sum cost of obtaining all parameters based on NGS-CR is < cost of alternatives | • Cost < pronghorn<br>• Increased spatial extent for kit fox<br>• Increased temporal frequency for pronghorn |

| | | | | |
|---|---|---|---|---|
| | • Increase in the temporal resolution of estimates versus alternatives | • Temporal extent and resolution of monitoring program<br>• Number of reliable parameters that can be obtained via NGS-CR versus alternatives<br>• Cost of obtaining parameters via NGS-CR versus alternatives | • Given a fixed cost for monitoring, area monitored is $\geq$ the area that could be monitored under current approaches<br>• Given a fixed cost for monitoring, estimates of demographic parameters can be obtained more often than current approaches | |
| 4. Ease of use | • Ability of a technician-level individual to implement sampling design | • Feedback from technicians on ease of data collection via standard Likert survey (1= strongly disagree to 5= strongly agree) | • Responses from Likert survey indicates agreement with ease of implementation of field protocol by a score ≥3.5. | • Yes |
| 5. Obtain estimates of occupancy and dynamic parameters (i.e., local colonization and extinction) via NGS-OM monitoring for kit foxes, at reduced costs relative to NGS-CR | • Parameter estimates for:<br>1. Proportion of area occupied<br>2. Colonization<br>3. Extinction<br>4. Species interactions<br>5. Costs | • Parameter estimates<br>• Costs of implementation | • Effective parameter estimates and inferences on species interactions<br>• Reduced cost relative alternative monitoring strategies | • Yes |
| **Qualitative Performance Objectives** | | | | |
| 6. Implementation of monitoring programs for kit fox and Sonoran pronghorn based on NGS-CR | • Consideration of implementing a NGS-CR monitoring program by federal/ state agencies or other organizations | • Records of interactions with persons responsible for managing focal species regarding implementation of monitoring programs based on NGS-CR | • Demonstrated interest and positive interactions about NGS-CR monitoring from federal/state agencies or other organizations and/or a Likert score ≥3.5. | • Yes |

*Description of each performance objective*

1. Improved monitoring protocol for kit fox and Sonoran pronghorn based on NGS-CR.

This performance objective evaluated whether or not a monitoring program based on NGS-CR provides more information (i.e., parameters characterizing population demographics and genetic health) when compared to currently utilized alternative approaches. This performance objective will be an important consideration for future implementation in that an improved monitoring approach would ideally provide more information to base management decisions than alternatives. This performance objective was important for subsequent analysis of cost-benefit in that it provided the number of parameters that can be estimated using NGS-CR versus alternatives, which were used in the calculation of the benefits of alternative approaches.

To evaluate whether the performance objective was met, we determined the number of parameters that could be obtained via monitoring programs based on NGS-CR versus alternative approaches. We obtained this information independently for each focal species. Depending on the spatio-temporal sampling design finalized in Year 3 of this project as well as the capture-recapture model used to estimate parameters, we quantified the number of demographic parameters that were estimated for each species using NGS-CR. Potential parameters obtained using Pollock's robust design include abundance, reproduction, and survival. While many capture-recapture models assume permanent emigration, this assumption is often violated. To account for this, Pollock's robust design allows for the estimation of the probability of temporary emigration, which can then be used to obtain unbiased population demographic parameters when temporary emigration occurs (Kendall et al. 1997). We also quantified any additional parameters important for population monitoring (e.g., occupancy, genetic diversity) that can be obtained using data collected for NGS-CR. The sum of these parameters was compared to number of parameters that are currently obtained for these two species using alternative approaches. If the number of parameters for each species was greater under a NGS-CR approach than from alternative monitoring efforts, then we considered this performance objective met.

2. Obtain reliable estimates of demographic parameters via implementation of NGS-CR monitoring protocol for kit fox and Sonoran pronghorn

Our second performance objective evaluated the precision of the abundance estimate obtained via NGS-CR by determining if the estimate had a coefficient of variation (CV) <10% of the expected value. As a general rule a CV <10% is ideal, but <20% indicates a precise estimate (White et al. 1982, Pollock 1990). To evaluate this performance objective, we estimated abundance after the revised sampling design was implemented in Year 3. We then estimated the variation in abundance (e.g., standard errors). We divided the parameter estimates by their standard deviations to obtain a CV. If the revised sampling design implemented in Year 3, representing the first year of a long-term monitoring program, resulted in a parameter estimate with a CV <10%, then we determined this performance objective had been met. We also evaluated whether our data would be useful for estimating survival, reproduction, genetic diversity, and genetic structure for both species.

3. Improve efficiency of current monitoring programs

The first two performance objectives were intended to establish that monitoring programs based on NGS-CR can provide reliable information for monitoring species of concern. An improved approach should also be one that can be implemented at large spatial scales, maintained for

extended periods of time, and provide estimates of population parameters at a fine enough temporal resolution (i.e., time between successive estimates) to be management relevant. Therefore this performance objective evaluated the efficiency of monitoring based on NGS-CR in terms of spatio-temporal extent and resolution, as well as the cost-benefit. To evaluate this performance objective, we recorded (1) the spatial extent to which parameter estimates apply, (2) the time between successive parameter estimates, and (3) the cost associated with obtaining the parameter estimates. This information was determined based on the revised sampling design implemented in Year 3 and was compared to the same information determined from alternative monitoring approaches currently implemented for both species. We compared the cost of obtaining each parameter for a given spatio-temporal extent and resolution based on current monitoring approaches versus NGS-CR individually and collectively. We considered this performance objective met if (1) the cost of obtaining each parameter based on NGS-CR is less than the cost of alternatives, (2) the sum cost of obtaining all parameters based on NGS-CR is less than the cost of alternatives, (3) for a fixed cost, the spatial extent to which parameter estimates apply is greater based on NGS-CR versus alternatives, and (4) for a fixed cost the frequency with which parameter estimates can be obtained more often than alternative approaches.

4. Ease of use

One of the potential benefits of monitoring programs based on NGS-CR is that field data can be collected easily by technician-level personnel, alleviating the need for extensive training often required for alternative monitoring strategies (e.g., live-capture, visual surveys, radio-telemetry, aerial surveys, etc.). Therefore, for managers and agencies responsible for implementing monitoring programs to adopt NGS-CR, it was important to demonstrate that the approach can be successfully implemented using technician-level individuals. To evaluate this performance objective, we collected responses of personnel tasked with collecting field data to a Likert-type qualitative survey with statements suggesting that for NGS-CR (1) field collection protocols were easy to follow, (2) required data could be collected under actual field conditions, (3) there were few situations encountered in the field which prevented data collection, and (4) a minimal amount of training and experience was required to collect field data. We used a 5-point scale that ranged from a score of 1 (strongly disagree) to 5 (strongly agree). Questions can be found in section 6 (Performance Assessment). We considered this performance objective met if responses to the survey based on implementation of the final protocol/design indicated that respondents agreed that implementation was easy and straightforward, which required a score $\geq 3.5$. We also evaluated whether the managers expressed an interest in continuing to employ NGS monitoring approaches, which they did at both installations (see #6 below).

5. Obtain estimates of occupancy and dynamic parameters (i.e., local colonization and extinction) via implementation of NGS-OM monitoring for kit foxes

As an alternative method to telemetry-based approaches for investigating species-habitat and interspecific interactions, NGS-OM can provide reliable information on occupancy for sensitive species. Occupancy modeling can incorporate covariate data (e.g., habitat, soil, distance to water) and provide information on landscape features that influence species occurrence (MacKenzie et al. 2006). Occupancy modeling can be further extended to investigate the influence that a dominant species (e.g., coyotes) has on the occurrence and distribution of a subordinate species (e.g., kit foxes) through co-occurrence models (Richmond et al. 2010) or through the use of

spatial replication (Lonsinger 2015). This is particularly relevant to the intraguild competition between kit foxes and coyotes, where it has been postulated that increases in coyote distribution and abundance at DPG are the result of increases in anthropogenic water sources (Arjo et al. 2007). While NGS-CR methods provide an effective and cost-efficient approach for estimating population demographics, additional information on the spatial dynamics of populations can be ascertained through NGS-OM with minimal additional costs. Thus, NGS-OM can increase the number of parameters estimated from NGS, may serve as a more affordable monitoring alternative to NGS-CR, and can be extended as needed to other regions or sensitive species. NGS-OM requires only DNA extraction and species ID of samples so costs are lower than for NGS-CR.

To evaluate this performance objective, we employed dynamic occupancy models to estimate parameters for detection, proportion of area occupied, colonization, and local extinction, and to generate 95% confidence intervals for each parameter. Additionally, we recorded (1) the spatial extent to which parameter estimates applied, (2) the time between successive parameter estimates, and (3) the cost associated with obtaining the parameter estimates. We compared the cost of implementing only a NGS-OM monitoring approach to only a NGS-CR approach. We also considered the impacts on overall cost that could be ascertained by employing a combination of molecular species identification and statistical classification tree identification. We considered this performance objective met if (1) we were able to obtain estimates of occupancy parameters, and if (2) the cost of implementing NGS-OM was lower than implementing NGS-CR monitoring.

6. Implementation of monitoring programs for Sonoran pronghorn and kit fox based on NGS

We anticipated that if all previous performance objectives were met, then agencies and personnel responsible for monitoring species of concern would view NGS (i.e., NGS-CR and/or NGS-OM) as a practical alternative and seek to implement monitoring programs based on this approach. Thus, the ultimate test of whether our demonstration was successful is whether NGS is adopted and implemented for future monitoring programs. We evaluated this qualitative performance objective by recording interactions with persons responsible for managing our focal species as well as other species of concern to DoD. We considered this performance objective met if interactions with stakeholders suggested a commitment to implementing future monitoring programs based on NGS.

# 4.0 SITE DESCRIPTION

## 4.1 SITE LOCATION AND HISTORY

We selected two species and demonstration sites for implementing monitoring programs based on NGS-CR. Our criteria for selecting demonstration sites included (1) an existing need for DoD to monitor the status of particular species of management concern, (2) it was difficult or costly to monitor these species using traditional approaches, (3) there had been successful implementation of monitoring programs based on NGS-CR for closely related taxa, (4) there was existing monitoring or research programs for target species that could be leveraged to provide additional information to evaluate NGS-CR monitoring programs, (5) a current relationships with DoD and other agency managers/biologists, and (6) geographic and taxonomic separation to demonstrate transferability. The two species and installations that met these criteria were kit foxes on DPG and Sonoran pronghorn on Barry M. Goldwater Range (BMGR), also planned for reestablishment on Kofa National Wildlife Refuge (NWR) and surrounding Yuma Proving Ground (YPG) in December 2012 (Figure 17).

Kit foxes occur on numerous military installations in the west (e.g., DPG, Nellis Air Force Range, YPG and BMGR, White Sands Missile Range, and Fort Bliss). They are classified as a Sensitive Species by the Bureau of Land Management and Forest Service, a Species of Concern in Utah, threatened in Oregon, and Endangered by the Colorado Division of Wildlife. The San Joaquin kit fox, the largest subspecies of kit fox, is found in California and is listed as endangered under the ESA; there is growing concern kit foxes will be petitioned for listing in other regions. In the long term, if a monitoring program can be implemented to evaluate the status of kit foxes and the effects of management actions, DoD may be able to assist in proactively preventing the species from being listed under the ESA and, thus, preclude subsequent restrictions imposed by listed species on DoD lands. Thus, there is a current need to monitor kit foxes and determine the effects of management and training actions on this species. Alternative methods for monitoring require physical capture and are expensive to implement, or provide only indices of abundance. We chose DPG as our focal installation to leverage current research on kit foxes (E. Gese, Utah State University and Robert Knight, DPG) that we used to both inform our sampling design and provide alternative estimates of abundance and survival based on telemetry monitoring (Arjo et al. 2007, Kozlowski et al. 2008). For example, Gese's current research has demonstrated that kit fox density on DPG was 0.05 foxes/$km^2$ and home ranges were 7–8 $km^2$ (Arjo et al. 2007). Also, during our 2012 and 2013 field seasons Gese's research group radio-tracked approximately 25 foxes on the DPG. This provided a comparison with which to evaluate our NGS-CR approach. Additionally, we garnered support and input for our proposed research from Robert Knight (Natural Resources Program Manager, U.S. Army DPG).

As a second demonstration, we developed a monitoring program based on NGS-CR for Sonoran pronghorn on BMGR and the surrounding area (i.e., Cabeza Prieta NWR). Historically, Sonoran pronghorn were relatively common in wide alluvial valleys of the Sonoran Desert (USFWS 2010). Widespread decline began in the mid- to late-1800s due competition with domestic livestock, fencing, and hunting which has reduced the current distribution to about 7.6 % of their original range (USFWS 2010). Sonoran pronghorn were federally listed as endangered in 1967

under the Endangered Species Preservation Act of 1966 and subsequently grandfathered in under the ESA of 1973 (USFWS 1998). Most of the current U. S. population resides on the southwestern portion of BMGR and adjoining Cabeza Prieta NWR (CPNWR). In a report to Congress, McCullough (2005) estimated $3.9 million was spent by DoD on Sonoran pronghorn studies and projects from 1995 to 2005 because of their endangered status. Additionally, this report concluded that "the diversion of time and energy for senior staff and officers and their consultations with numerous other agencies to ensure compliance with [Sonoran] Pronghorn protection is a frequent occurrence that causes the focus to shift to endangered species concerns rather than the military's mission" (McCullough 2005:10). The Recovery Plan for Sonoran pronghorn states the species will be considered for downlisting when one self-sustaining population of an estimated population of 300 adults has been established in the U.S. for a minimum of 5 years, and a least one other self-sustaining population has been established in the U.S. (USFWS 1998). To achieve these goals, the USFWS proposed establishing two additional wild populations to occur, at least partially, on the eastern portion of BMGR (i.e., east of Arizona State Highway 85) and YPG (USFWS 2010). To determine the success of these management actions, and thus facilitate downlisting, it is in USFWS's and DoD's joint interest to efficiently monitor the current and reestablished populations. Presently, Sonoran pronghorn are monitored based on aerial counts that provide estimates of abundance biennially. While this approach provides robust estimates, it is costly and does not provide information on survival, reproduction or genetic diversity. Furthermore, the high costs prevent managers from obtaining estimates more frequently than every other year. Thus, there is great potential for monitoring based on NGS-CR to provide critical information while doing so at reduced cost, especially in the future when other populations become reestablished and the inhabited range expands. We implemented a targeted sampling approach for Sonoran pronghorn because they are known to use anthropogenic water sources (i.e., drinkers) that have been established for them especially during the dry season (Morgart et al. 2005).

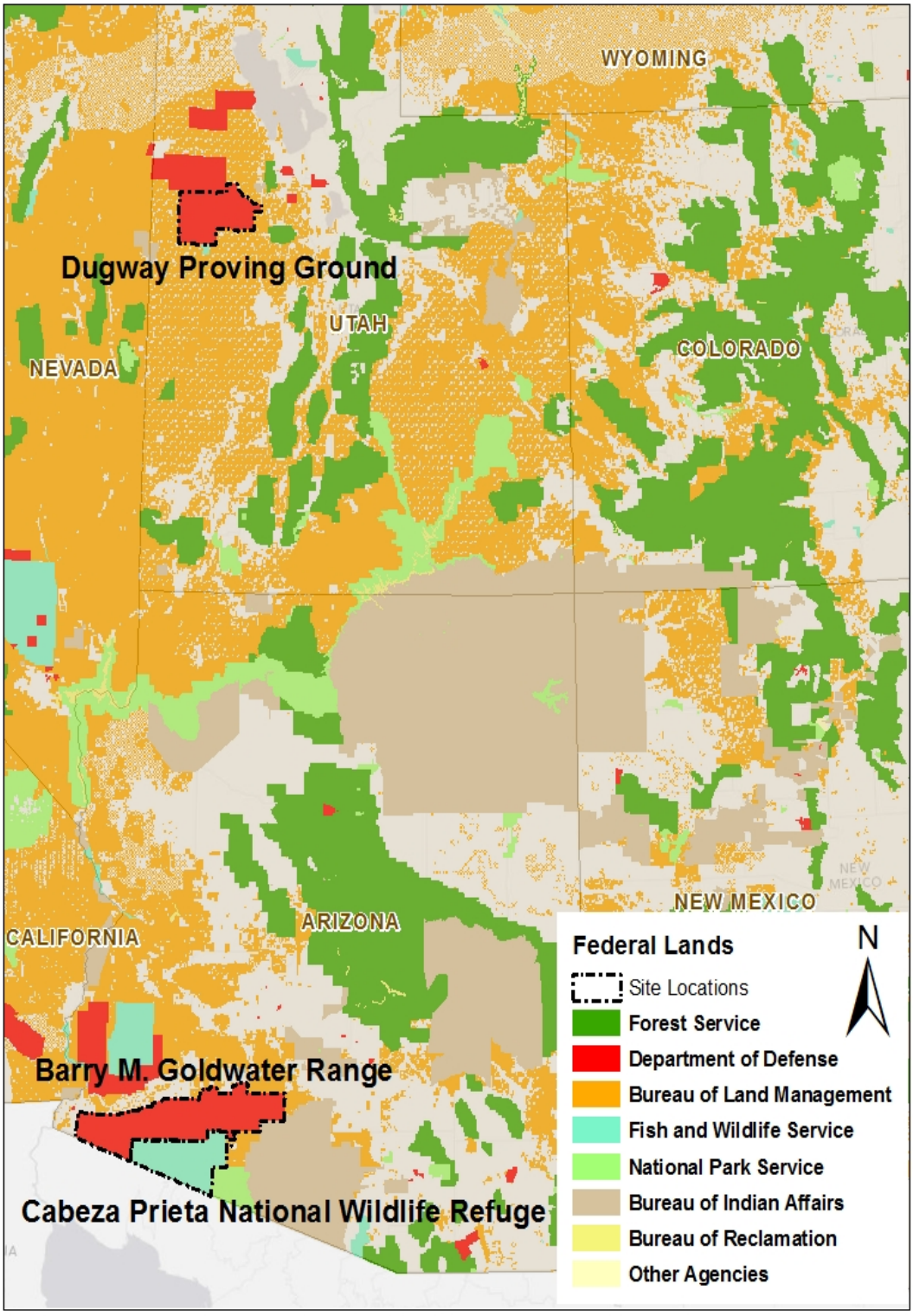



**Figure 17. Location of demonstration sites, Utah and Arizona.**

### 4.1.1 Dugway Proving Ground (DPG), Utah

The United States Army's DGP, located at the southern end of Utah's Great Salt Lake Desert, is approximately 130 km southwest of Salt Lake City, Utah and 65 km south of Interstate 80 (DPG 2007). The western boundary of DPG lies approximately 29 km east of the Nevada border. Extending nearly 84 km east-west by 48 km north-south at its widest points, DPG encompasses 798,214 acres (DPG 2007). In response to the bombing of Pearl Harbor, DPG was founded in 1942 by presidential action as an essential facility for testing both weapons and defenses of chemical and biological weapons of mass destruction and for military training. Initially only 126,720 acres, the size of DPG has increased significantly.

Currently, DPG is among the elite U.S. military installations and maintains a mission of providing premier training, testing, and evaluation for our nation's leading military forces. Testing and training related to defenses against chemical and biological weapons, remediation technology, battlefield munitions, smokes and obscurants, and survivability of military equipment in chemical or biological conditions, as well as specialized collective trainings including live-fire scenarios, all occur on DPG (DPG 2007).

Changes to the habitat on DPG and surrounding areas that may influence the distribution and abundance of kit foxes include increases in fire frequency and severity (DPG 2007), juniper (*Juniperous osteosperma*) encroachment, and the development of anthropogenic water sources (Arjo et al. 2007). The altered fire regime has resulted in large monocultures of invasive cheatgrass (*Bromus tectorum)* as well as the spread of other invasive species, such as tumbling mustard (*Sisymbrium altissimum*) and Russian thistle (*Salsola iberica*), among others. The introduction of anthropogenic water to the landscape in the 1970's may have facilitated the increase in coyote settlement on DPG, increasing both competition and predation risk for kit foxes.

Training and testing on DPG were expected to have limited impacts on the demonstration at DPG. Surveys associated with the demonstration plan involved transects along low to moderate use roadways. We anticipated the following impacts to the demonstration: (1) negligible temporal shifts in sampling to avoid active military training or testing, and (2) spatial avoidance of closed impact areas or restricted areas without prior approval. Wig and Granite Mountains will represent the western extents of surveys associated with the demonstration in the northern and southern portions of DPG, respectively. The majority of closed impact areas and restricted areas are found west of these mountains. Only a single impact area (i.e., the White Sage Impact Area) had to be avoided within our sampling frame. We occasionally encountered live-fire training or similar activities that precluded short-term use of an area. Through coordination efforts with DPG, we were able to access all survey sites within 48 hours of our intended survey date. Increases in vehicle traffic (both on DPG and on neighboring federal lands) in summer relative to winter impacted survey efforts that occurred along larger gravel roadways. Still, the majority of transects were located along two-track roadways and our sampling intensity (i.e., repeat surveys) likely mitigated for this effect.

### 4.1.2 Barry M. Goldwater Range, Arizona

The Barry M. Goldwater Range in southwestern Arizona is a 1.7 million acre training range used by U.S. and allied pilots for air-to-ground and air-to-air training missions. Established in September 1941, the range totaled 1.1 million acres divided into the western section, originally called the Yuma Aerial and Gunnery and Bombing Range, and the eastern section, known as the Gila Bend Gunnery Range and later the Ajo-Gila Bend Gunnery. During World War II, the range was expanded to 2.1 million acres and was the largest single engine advanced flying training facility in the U.S. with more than 17,000 pilots training there during the war years (LAFB 2012a). Post-World War II, additional range expansions were necessary to accommodate jet fighters. With the closure of Luke Field from November 1946–February 1951, the range was renamed the Williams Bombing and Gunnery Range. Escalating conflict in Korea increased demand for fighter pilots and Luke Air Force Base (LAFB) was established in February 1951. LAFB took over management of the eastern and western sections and the range was again renamed, now the Luke Air Force Range, in 1963. In 1986, Congress renamed the range again in honor of Arizona Senator Barry M. Goldwater.

Numerous expansions and reductions over the years have brought the range to its current size. Today, the eastern portion of the range is under the management of LAFB Range Management Office, while the western portion is managed by Marine Corps Air Station Yuma. Active duty, Guard, and Reserve Pilots from the Army, Navy, Marine Corps, and Air Force use the range. BMGR is the third largest tactical aviation range in the US with nine air-to-ground and two air-to-air ranges, which allow over 50 aircraft to carry out simultaneous training missions (LAFB 2012b and 2012c).

While live bombs are used on a portion of the range, the majority of the range sees low flying aircraft with no munitions deployment. These large areas with little human presence are inhabited by a variety of endangered and protected species including flat-tailed horned lizard (*Phrynosoma mcallii*), lesser long-nosed bat (*Leptonycteris yerbabuenae*), cactus ferruginous pygmy owl (*Glaucidium brasilianum cactorum*), and Sonoran pronghorn (Bagne and Finch 2012). Extensive monitoring programs are in place to ensure minimal disturbance of these endangered species.

Due to safety concerns associated with live-fire training, public access is by permit only and is strictly regulated. Access for NGS-CR sampling was restricted to either “No-Fly” weekends or to early morning, pre-arranged visits, which was coordinated with Chiulista Services contract biologists from the BMGR.

### 4.1.3 Cabeza Prieta National Wildlife Refuge

CPNWR is the third largest wildlife refuge in the lower 48 and is administered by the USFWS. In 1939 following a multi-year, statewide campaign by the Boy Scouts of Arizona to protect desert bighorn sheep, President Roosevelt created the Cabeza Prieta Game Range—now known as the CPNWR—and in 1990, ninety percent of the refuge was designated wilderness to further protect the area. The area was once inhabited by the Tohono O'odham and Sand Papago peoples

and is rife with Native American artifacts. The refuge is part of the largest interconnected, protected area in the lower 48 and Mexico, which includes BMGR, CPNWR, Organ Pipe Cactus National Monument, and the Pinacate Biosphere Reserve in Mexico, and is home to more than 400 plant species and over 300 wildlife species. Construction of a border fence along the international border in an attempt to reduce illegal traffic from Mexico has severed connections between the U.S. and Mexico populations of pronghorn. The border fence is expected to block gene flow between the two populations.

The refuge is open and accessible most of the year upon obtaining a free permit from the CPNWR office in Ajo, AZ; however, parts of the refuge are off-limits due to military flight training missions. Sample collection for this project was coordinated with trips made by AZGFD and USFWS personnel to minimize number of trips to drinkers and impact to pronghorn.

## 4.2 SITE CHARACTERISTICS

### 4.2.1 Dugway Proving Ground (DPG), Utah

DPG is located at the southern end of Lake Bonneville, a prehistoric lake that once covered much of the Great Basin. The influence of Lake Bonneville is most evident in the western portion of the installation, which is characterized by salt flats as a consequence of its lake bottom origins. Located within the Great Basin, DPG and the surrounding areas are characterized by the typical basin and range formations (DPG 2007). Extension of the earth's crust during the Cenozoic period along parallel faults has resulted in low-lying basins bounded by north-south running mountain ranges (Thompson and Burke 1974). Elevations at DPG range from 1228 m to 2154 m on Granite Peak (DPG 2007). Characterized as a cold desert, DPG and surrounding areas experience cold winters and moderate summers, with the coldest and warmest months being January (average high = 4°C, average low = -10°C) and July (average high = 36°C, average low = 15°C), respectively. Average annual rainfall is approximately 21.9 cm with the greatest rainfall occurring in the spring from March to May (DPG 2007).

A range of Great Basin habitats are present on DPG. The most abundant habitat type is Cold Desert Playa, which covers nearly half of the installation and is predominantly found in the western and northern portions of the proving ground, a result of Lake Bonneville's influence. The eastern portion of the installation is dominated by Cold Desert Chenopod Shrubland and vegetated dunes, along with non-native invasive grasslands which dominate following wildfire activity. The higher elevations along the slopes of the mountains are characterized by arid shrubland and open woodland at the highest elevations. Unvegetated dunes and wetland habitats are available to a lesser extent across DPG (DPG 2007). Habitats in the adjacent Skull Valley and surrounding mountains are similar, but a more active fire history in the valley's basin has resulted in a greater frequency of non-native invasive grasslands (i.e., cheatgrass).

Water availability for wildlife has increased dramatically on DPG. Historically, there were believed to have been only nine water sources to support wildlife and these natural springs were distributed at higher elevations (Arjo et al. 2007). The development of four sewage lagoons and the installation of 11 guzzlers for upland game birds (5) and big game (6) have greatly increased

the available water. Additional water is available from collection ponds for developed facilities and irrigation. Water availability in the adjacent Skull Valley is abundant with active grazing practices providing water for livestock. Active water manipulations including exclusion of wildlife from select lagoons and guzzlers were ongoing during our surveys and were considered in our sampling design, but were not anticipated to impact the demonstration surveys.

In addition to kit foxes, DPG accommodates a range of species that compete with and/or prey upon the kit fox. Intraguild competition and/or predation can occur from cougars, bobcats, American badgers (*Taxidea taxus*), coyotes, and red foxes. Likely facilitated in part by the increase in available water, coyote populations have increased since the 1950's when researchers (H. Egoscue) indicated that they were rare in the area (Arjo et al. 2007). Red foxes have been detected by multiple researchers on the installation, but are suspected to be rare. The habitat requirements of mountain lions and bobcats overlap to a much lesser extent with kit foxes. In addition to kit foxes, we detected coyotes, red foxes, bobcats, mountain lions, and domestic dogs during the demonstration.

The ecology of kit foxes was first investigated on DPG when Egoscue initiated studies in 1951 (Egoscue 1956, 1962). More recent research has focused on den selection (Arjo et al. 2003) and the interaction of kit foxes and coyotes (Arjo et al. 2003, Kozlowski et al. 2008, 2012). Ongoing research led by Utah State University (USU) continues to explore the interaction between kit foxes and coyotes while research led by Brigham Young University investigates the use of free-standing water by kit foxes (and other species). Demonstration surveys were conducted in collaboration with USU researchers. We utilized information from concurrent USU canid monitoring efforts based on traditional monitoring techniques (e.g., live-capture-recapture, radio-telemetry, scat deposition surveys) to make comparisons and evaluate the efficiency of NGS-CR and NGS-OM approaches.

### 4.2.2 Barry M. Goldwater Range, Arizona

BMGR lies within in the basin and range lowlands region of southwestern Arizona. The region is characterized by wide alluvial valleys divided by fault-block mountains. There are no weather stations on BMGR; however average summer high temperatures in nearby Gila Bend are over 38°C (INRMP 2003). Average temperatures in winter range from 4 °C to 24 °C. April–June is the dry season as most precipitation falls in winter and late summer with monsoon rains. Rainfall varies dramatically and declines from east to west; average annual precipitation for the range is ~21.7 cm on the southeastern edge and ~11 cm annually on the northwestern edge of the range (INRMP 2003). Higher elevation areas see higher amounts of precipitation with up to 28 cm annually (INRMP 2003). Climate shifts in the past 25 years have led to warmer, drier conditions (Weiss and Overpeck 2005, Kimball et al. 2010) and winter rains, which once started in October, often now arrive in December (Kimball et al. 2010).

Vegetation on the BMGR is characterized by Arizona Upland and Lower Colorado River Valley subdivisions (Brown 1982). Scrub vegetation communities vary throughout the range with the topography, elevation, and presence/absence of washes, and thus frequency and amount of water. Main waterways are host to ironwood (*Olneya tesota*), blue palo verde (*Parkinsonia floridum*),

jojoba (*Simmondsia chinensis*), and mesquite (*Prosopis velutina* and *P. glandulosa*), bordered by open creosote (*Larrea tridentata*) and white bursage (*Ambrosia dumosa*) flats (USFWS 1998). Upland vegetation consists of foothill palo verde (*Parkinsonia microphyllum*), catclaw acacia (*Acacia greggii*), chain fruit cholla, teddy bear cholla (*Cylindorpuntia bigelovii*), buckhorn cholla (*C. acanthocarpa*), and staghorn cholla (*C. versicolor*) (USFWS 1998). Mesquite-creosote habitat and mixed cacti/palo verde bajadas, which extend from the base of the hills into the floodplain and typically form when alluvial fans meet, are also present (USFWS 1998). Other upland vegetation includes ocotillo (*Fouguieria splendens*), elephant tree (*Bursera microphylla*) and other cacti and shrub species (Shreve and Wiggins 1964). Barrel and organ pipe cacti are found less commonly (Brown 1982). Other natural communities include desert tinajas/springs, valley bottom floodplain, dune complex/endemics, and salt desertscrub regions are also found in the region (Shreve and Wiggins 1964).

Natural surface water in the region is limited (USFWS 2010, Bagne and Finch 2012). Multiple artificial drinkers have been installed to provide water to Sonoran pronghorn, sheep and other species, and three combination water/feed sites exist on the range to supplement natural forage for pronghorn. Alfalfa and water are hauled in weekly by AZGFD or USFWS personnel during the dry months. Feeding and watering typically begins and April or May and ends in October or November depending on annual rainfall amounts (USFWS 2015).

BMGR lies within the range of multiple federally threatened or endangered mammalian species including Sonoran pronghorn and the lesser long-nosed bat, as well as cactus ferruginous pygmy owl, a State of Arizona species of greatest conservation need, and the California leaf-nosed bat (*Macrotus californicus*) and Mexican long-tongued bat (*Choeronycteris mexicana*), which are both designated as *federal species of concern* and *species of greatest conservation need* in the state of Arizona (Bagne and Finch 2012). Pronghorn have been shown to be attracted to areas disturbed by military use due to potential pooling of water in bomb craters, ease of detecting predators, and increased forage due to disturbance and fires (Hervert et al. 1997, Krausman et al. 2005). In areas known to have pronghorn, contract biologists from Chiulista Services, Inc. conduct daily visual scans for pronghorn prior to bombing missions. If pronghorn are located within 5 kilometers of a target, the mission is called off or redirected to a different area (LAFB 2012b).

While this demonstration plan focused on Sonoran pronghorn, we made every effort to minimize impact on all other wildlife species.

### 4.2.3 Cabeza Prieta National Wildlife Refuge, Arizona

CPNWR lies within in the basin and range lowlands region of southwestern Arizona, and the region is characterized by wide alluvial valleys divided by fault-block mountains. CPNWR covers approximately 2600 $km^2$, encompasses seven mountain ranges, and the majority is designated wilderness. Lying adjacent to BMGR, climate and rainfall patterns are generally the same. The CPNWR falls on the boundary of the Lower Colorado River Valley subdivision and is one of the hottest and driest regions of North America. From June to October, it, temperatures can be above 32–38 °C for over 100 days in a row (USFWS 2011). Winter temperatures average

0 °C–24 °C. Vegetation is similar to the BMGR and is dominated by creosote bush, bursage, saltbush, with ironwood, blue palo verde, jojoba, and mesquite in areas with more water. A variety of cholla, saguaro, organ pipe, and barrel cacti are found here as well.

Quitoboquito Spring, located on Organ Pipe Cactus National Monument, is the only naturally occurring year-round water source in current Sonoran pronghorn range and occurs in close proximity to a busy highway and in an area of high illegal activity related to the border (Slone 2011). Numerous artificial water sources have been developed by improving old livestock water tanks and building new catchment systems and temporary waters. These drinkers exist on the refuge specifically to supplement water for bighorn sheep and Sonoran pronghorn, but remote cameras installed at the water sites show use of drinkers by coyotes, eagles, vultures, mule deer, and other birds and mammals as well (Pers. Comm. J. Atkinson). Forage enhancement plots have been constructed to promote growth of natural vegetation by irrigating areas adjacent to some drinkers.

Following a severe drought in 2002 which caused high rates of pronghorn mortality, a semi-captive breeding pen was constructed to facilitate pronghorn recovery (Otte 2006). The one-square mile facility is double fenced and electrified to keep pronghorn in and predators out. Bucks are moved between the pens and occasionally a wild buck is captured and introduced into the pen to increase genetic diversity (USFWS 2015). Drinkers in the pen are filled from a well and portions of the pen are irrigated to encourage growth of natural forage. Additionally, pronghorn are fed alfalfa and supplemental vitamin pellets approximately every other day. In 2006, two yearling males were the first individuals to be released from the captive pen. Successful fawn recruitment into the captive pen has allowed for additional releases in subsequent years. A total of 128 pronghorn have been released from the pen into the wild population, most of which are marked with ear-tags and/or radiocollars to allow for continued monitoring (USFWS 2015).

Management of Sonoran pronghorn on BMGR is led by USFWS with support from AZGFD. Current surveys and monitoring of wild pronghorn on the refuge consist of locating radio-collared individuals opportunistically from the ground, bi-monthly monitoring flights, and a biennial population count (USFWS 2015). Demonstration surveys were be conducted in collaboration with USFWS and AZGFD researchers. Current efforts to monitor Sonoran pronghorn were evaluated and compared to the results of the NGS-CR demonstration.

# 5.0 TEST DESIGN

## 5.1 CONCEPTUAL TEST DESIGN

The primary demonstration included four main stages spanning three years (see Figure 2). The first stage occurred in year one and included three main components: (1) an evaluation of fecal deposition rates in each study area, (2) development of species identification and individual identification methods for each species, and (3) a DNA degradation experiment in each study area that evaluated PCR success and genotyping error rates for species and individual ID over different fecal sample exposure times ranging from 1 day to 4 months. The second stage of the demonstration in year two included a pilot implementation of the NGS-CR population estimation methods over two primary sessions followed by power analyses to determine the optimal spatio-temporal study design for the final demonstration in year three. The third stage included implementation of the optimized NGS-CR monitoring approach in each study area. The fourth stage of the demonstration involved cost-benefit analyses of the demonstrated NGS-CR approach compared to alternative monitoring methods. These four main stages provided the data needed to address our performance objectives.

Field sampling was conducted within a Pollock's robust design framework,. Extensions of Pollock's robust design incorporate genotyping errors associated with NGS-CR (Lukacs et al. 2009) and partitioning of recruitment into gains from reproduction versus immigration (Sandercock 2006). Furthermore, data collected within a Pollock's robust design can be analyzed under alternative capture-recapture models. At each demonstration site and with each species, appropriate application of Pollock's robust design accounted for the ecology of the target species. To this end, sampling of kit foxes and Sonoran pronghorn was accomplished utilizing two primary periods distributed temporally based on the target species. The implementation of Pollock's robust design, including description of the temporal distribution of primary and secondary periods, is discussed in greater detail in section 5.5.1 (kit foxes) and 5.5.2 (Sonoran pronghorn).

Throughout the demonstration, we collected and compiled data on the cost of implementation that was used to directly compare our approach with alternative monitoring approaches. We explored ways to further reduce costs and demonstrated an effective tool for long-term population monitoring including investigating the effectiveness of subsampling strategies, evaluating new NGS-CR models designed to reduce lab costs, and employing a power analysis to evaluate the level of effort necessary to achieve desired levels of precision. In addition to the proposed non-spatial Pollock's robust design and 'capture with replacement' (CAPWIRE) models designed, we also compared these abundance estimators to spatially-explicit models for kit foxes, which are becoming increasingly popular and provide estimates of density.

For kit foxes, we also demonstrated the utility of employing NGS-OM approaches to quantify the spatial dynamics of kit foxes and to investigate the role of habitat and landscape features (e.g., water availability, shrub cover), as well as intraguild predators (i.e., coyotes), on patterns of kit fox space use. Through this application of NGS-OM, we demonstrated how spatial replication can be used in place of temporal replication to both increase the spatial extent of monitoring and investigate the influence of interspecific interactions when a co-occurrence modeling framework

was impractical. Additionally, we compared statistical classification approaches to field identification (ID) of carnivore scats and evaluated rates of scat removal, to further improve efficiency and inform future noninvasive monitoring of carnivore species.

## 5.2 BASELINE CHARACTERIZATION AND PREPARATION

*Kit Fox*

We chose DPG as our focal installation to leverage concurrent research on kit foxes (E. Gese, USU and Robert Knight, DPG) that could be used to both inform our sampling design and provide alternative estimates of abundance and survival based on telemetry monitoring (Arjo et al. 2007, Kozlowski et al. 2008). For example, Dr. Gese's research demonstrated that the density of kit fox on DPG was 0.05 foxes/km$^2$ with home range sizes ~7–8 km$^2$ (Arjo et al. 2007). Scat deposition surveys conducted as part of Dr. Gese's research provided us with baseline data on the number of scats we could expect to encounter from both kit foxes and other intraguild species; estimates from these surveys indicated that we could expect to encounter 0.7–-1.0 kit fox scats/km/2 weeks. Also, during our 2012 and 2013 field seasons, Dr. Gese's research group tracked approximately 25 telemetered kit foxes on DPG. This provided a comparison with which to evaluate the efficacy of our approaches based on NGS.

*Sonoran Pronghorn*

Sonoran pronghorn were federally listed as endangered in 1967 under the Endangered Species Preservation Act of 1966 and subsequently grandfathered in under the ESA of 1973 (USFWS 1998). Most of the current U. S. population resides on the southwestern portion of BMGR and adjoining CPNWR. Presently, Sonoran pronghorn are monitored based on aerial counts that provide estimates of abundance biennially. While this approach provides robust estimates, it is costly and does not provide information on survival, reproduction or genetic diversity. Furthermore, the cost prevents obtaining estimates more frequently than every other year.

Two aspects of Sonoran pronghorn ecology and management were particularly relevant to our demonstration. The first is that Sonoran pronghorn are known to use anthropogenic water sources (i.e., drinkers) especially during drier times of the year (Morgart et al. 2005). Using this information, we designed our spatial sampling to collect feces at drinkers currently distributed throughout their range and this provided an efficient method for collecting sufficient sample sizes while minimizing travel and collection time. The second aspect is the maintenance of a captive population of Sonoran pronghorn at CPNWR. We utilized this captive population for our degradation study to collect feces $\leq$ 24 hours old to set up an experiment examining DNA degradation rates (See Section 2.2). This captive population has been the focus of analyses to evaluate the genetic profiles and reproductive success of the founders by the research group of Melanie Culver (University of Arizona). DNA samples (blood) collected by this research group from known age individuals during annual capture operations conducted by AZGFD and USFWS were shared with us for use in distinguishing age class from morphometric measurements of fecal pellets (See Section 2.2). We additionally used five microsatellite loci designed by this research group for pronghorn individual ID (Munguia-Vega et al. 2013).

## 5.3 DESIGN AND LAYOUT OF TECHNOLOGY AND METHODOLOGY COMPONENTS

*Laboratory Methodology and Equipment*

All laboratory analyses were conducted at the Laboratory for Ecological, Evolutionary and Conservation Genetics in College of Natural Resources at the University of Idaho. This facility is directed by PI Waits and includes all specialized genetics equipment needed.

*DNA extraction*

DNA extraction was conducted in a facility dedicated to low quantity DNA sources that is designed and managed to minimize the possibility of contamination. DNA was extracted from the fecal samples using the QIAamp DNA Stool Mini Kit (Qiagen, Inc.) and standard centrifuges. One negative control was included in each extraction to monitor for contamination of reagents.

*Species ID*

Species-specific polymerase chain reaction (PCR) primers were designed to conduct species identification of fecal samples. PCR set up was conducted in a facility dedicated to low quantity DNA sources and a negative control will be included to monitor for contamination. PCR was conducted on a BioRad Tetrad system.

We had already developed methods to detect kit fox DNA using a mitochondrial DNA fragment analysis test (Onorato et al. 2006, De Barba et al. 2014). We designed a similar test to distinguish pronghorn fecal pellets from mule deer (Woodruff et al. 2014). Fragment analysis and sequencing products were visualized using a 3130xl DNA Sequencer (Applied Biosystems) and allele sizes were scored using Genemapper 3.7 (Applied Biosystems).

*Individual ID*

Individual identification was conducted using nuclear DNA microsatellite analysis at 6–9 loci for kit foxes and 7–10 loci for pronghorn. The number of microsatellite loci needed to distinguish individuals depends on the level of genetic diversity (# alleles and heterozygosity) in the study population and the degree of relatedness among individuals. We chose a number of loci that ensured an observed probability of identity of zero and a theoretical probability of identity less than 0.01. The Waits laboratory has previously developed microsatellite PCR multiplexes for fecal DNA analysis of multiple canids including coyotes, gray wolf, red wolf (*C. rufus*), and swift fox (Kitchen et al. 2005, Adams and Waits 2007, Stenglein et al. 2010a), and these multiplexes were adapted for use in kit foxes. Sex identification primers had not previously been developed for kit foxes, but we adapted and successfully employed sex identification primers originally designed for red foxes (Berry et al. 2007). We have also developed microsatellite PCR multiplexes for 19 microsatellite loci for pronghorn (Byers and Waits 2006, Dunn et al. 2010) and have demonstrated that these loci can be used to genotype fecal pellets of pronghorn (Dunn et al. 2010). Sex identification primers had also not previously been developed for pronghorn. We again adapted and successfully employed sex identification primers originally designed for

other ungulates (Brinkman and Hundertmark 2009). Each sample was amplified twice using the identified microsatellite PCR multiplexes. We included primers for sex identification in the microsatellite PCR to efficiently obtain individual and sex ID in a single PCR reaction. The PCR products were separated by size with the Applied Biosystems 3130xl capillary machine, scored with the corresponding software Genemapper 3.7, and verified individually by eye. A negative control was included in all PCRs to test for contamination. If samples failed to amplify at <50% of the loci they were discarded from further analysis to remove low quality, error prone samples from the dataset.

To obtain a consensus genotype at each locus, we required an identical result across two PCRs for heterozygotes and additional replicates were conducted when the first two PCRs did not agree. An allele was not recorded in a consensus genotype unless it was observed twice. For homozygotes, we required 3 matching genotypes. This process of testing and evaluating was repeated until a consensus genotype was obtained at the loci to meet the minimum matching criteria of 0.01 P(ID)sibs (Waits et al. 2001).

Completed genotypes were then analyzed using the software GenAlEx6 (Peakall and Smouse 2001) or the ConGenR script (Lonsinger and Waits 2015), both of which pair matching samples and display near matches (i.e., those samples that differ at only one or two loci). Each group of samples that differed at one or two loci was evaluated in more detail to verify that they were indeed unique individuals. In DNA-based mark-recapture studies, single-capture individuals can result from an artifact of genotyping errors and greatly inflate population estimates. Therefore, an additional statistical analysis was performed to determine the confidence in the genetic results from single-capture samples. The software RELIOTYPE (Miller et al. 2002) was used to estimate the microsatellite genotyping error rate for each of these samples and to evaluate the reliability of the final consensus genotype given the estimated error rate and the allele frequencies in the population. In this analysis, the threshold was set at greater than or equal to 95% reliability. Any individuals that fell below the threshold were subjected to further testing in order to ensure accuracy, thereby increasing reliability rates above the threshold. At the completion of these analyses, each sample with a completed consensus genotype had a unique identification number that was used in subsequent capture-recapture models.

*Field Methodology and Equipment*

At DPG, our collaborators from USU had already identified and established 15 random 5 km transects. We retained these 15 transects, but aimed to increase the spatial extent for both NGS-CR and NGS-OM monitoring. We utilized Geographic Information Systems (GIS) and Global Positioning System (GPS) technologies to select and identify additional kit fox survey locations. For NGS-CR, 15 random sampling transects were selected using a combination of ArcGIS (ESRI, Redlands, CA 92373) and the Geospatial Modeling Environment (Beyer 2012). The Geospatial Modeling Environment was employed to randomly select cells for sampling that were similar in size to the home range size of kit foxes at DPG. Randomly selected cells were projected in ArcGIS, road layers in and around DPG were used to identify and delineate transects within each selected cell, and transect start and end points were identified. Similarly for NGS-OM, we used ArcGIS the Geospatial Modeling Environment to randomly select 60 sites (each 6.25km$^2$) from the study area, excluding sites with which already contained any portion of the

longer NGS-CR transects. Randomly selected sites were projected in ArcGIS along with road layers, and we delineated four 500 m transects within each selected site.

Reference points associated with all transects were uploaded directly into handheld GPS units and used to navigate to transect starting and ending points. While conducting surveys, researchers utilized GPS units to record the spatial locations of all encountered carnivore scats. These sample locations were downloaded daily and directly imported in an ArcGIS layer to eliminate errors associated with entering data manually. Projected sample locations, overlaid in ArcGIS with habitat and landscape layers (e.g., digital elevation model, habitat, soil, water sources, etc.), were then used to extract covariate data associated with each location, providing additional information that informed and improved estimates of population demographics and occupancy.

## 5.4 FIELD TESTING

Our main optimization stage was phase one (Year 1), where we evaluated fecal deposition rates and DNA degradation rates. For our sampling designs in year 2 and year 3, it was important to estimate the rate that fecal samples would accumulate along potential sampling transects (for kit foxes) and at the concentrated use areas (watering and feeding sites for pronghorn). After obtaining these estimates, we were able to estimate how many scats would be deposited per unit area per unit time. This information was essential to determining the required spatial and temporal sampling in year 2 and year 3.

We describe our pilot studies in Section 2.2. Here we focus on implementation of Year 2 and Year 3 of the study.

*Kit fox*

For both NGS-CR and NGS-OM, we implemented kit fox monitoring during two primary sampling periods, winter and summer, during which we assumed the population was closed both geographically and demographically. We selected winter and summer sampling seasons to align with periods preceding breeding and juvenile dispersal, respectively. The identification of these time periods was accomplished in collaboration with researchers from USU, who had been monitoring kit foxes via radio-telemetry and could help inform and refine the primary sampling periods.

The timing of winter sampling was intended to characterize the breeding population (i.e., those individuals surviving through winter and which had the opportunity to reproduce). Consequently, this population was expected to be have lower abundance (and potentially lower occupancy rates) than summer populations. An important consideration of winter sampling was to ensure sampling was complete prior to the initiation of natal denning, at which time females may have decreased availability for capture (Ralls et al. 2010).

Summer sampling was intended to characterize the population following reproduction. To this end, it was important that sampling occur within the narrow window during which juveniles (i.e.,

pups) were actively hunting with their parents (i.e., after emergence from the natal den) and prior to dispersal. Summer populations were expected to have the highest annual abundance (and potentially occupancy) due to the inclusion of adults and juveniles, many of which would not survive through to the following winter sampling event.

Within primary sampling sessions, the number of NGS-CR secondary sampling sessions (or occasions), and the duration of time between occasions was informed by our pilot studies (see Section 2.2; Lonsinger et al. 2015a) and subsequent power analyses. In year 2, we considered the length of transect that could be effectively surveyed and the observed scat accumulation rates from the pilot study. We then estimated the number of surveys that would be required to collect ~200 kit fox samples, a value that we expected to be approximately three times the number of individuals in the study area (Solberg et al. 2006). The interval length (i.e., the duration of time between the clear and a survey, or between sequential surveys of the same transect), was informed by our optimization scheme (Figure 4), aimed to reduce the overall cost per successful sample (Figure 9), and considered the simultaneous sampling of coyotes (see Section 2.2; Lonsinger et al. 2015a). For year 3, we adjusted the number of occasions based on the results of a power analysis (see Section 5.5.1), with the goal of achieving a CV ≤10% for abundance estimates; sampling interval remained the same.

*Pronghorn*

We implemented Sonoran pronghorn monitoring during a single primary sampling period in May and June each year during which we assumed both geographic and demographic population closure. Timing of our sampling season was post-fawning to enable sampling of fawns. Additionally, the sampling period coincided with the hot dry months when pronghorn are using drinkers and supplemental feed sites. The identification of these sampling periods was accomplished in collaboration with managers from USFWS and AZGFD to also minimize pronghorn disturbance as they are visiting drinkers weekly during this time.

Our pilot studies (see section 2.2. Woodruff et al. 2014, 2015) informed the number and sampling interval for secondary sampling sessions (or occasions), and subsequent power analyses. For pronghorn, we collected feces during 3 secondary periods separated by 7-day intervals during 2 primary sessions in Year 2, the first in May–June and the second in October–November corresponding to times when pronghorn were gathered at drinkers. Between Year 2 and Year 3, we conducted a power analysis (see next section) based on these results to inform year 3 sampling and implemented the spatio-temporal sampling design to achieve a CV <10% in the parameter estimates. Per results of the power analysis, we did not change the sampling design in Year 3; however, we did sample during only a single primary period (June) due to the paucity of samples collected during the fall session in Year 2.

## 5.5 SAMPLING PROTOCOL

### 5.5.1 Dugway Proving Ground, Utah

Accurate and precise estimates of population parameters are necessary to effectively monitor and conserve sensitive species. The kit fox, one of the smallest canids in North America, is a rare and elusive sensitive species of interest to the DoD. Kit foxes are nocturnal and utilize burrows year-round to provide relief from predation and climatic extremes. These behavioral adaptations make kit foxes difficult to detect through cost-effective survey techniques such as spotlighting and scent stations (Schauster et al. 2002, Dempsey et al. 2014). As a result, use of these cost-effective techniques has resulted in imprecise population estimates that are able to detect only large changes in population parameters (Warrick and Harris 2001). Previous efforts to achieve precise estimates of kit fox population parameters at DPG include traditional capture-recapture techniques, which are expensive and time consuming, and often lacked sufficient sample size to effectively estimate abundance. We evaluated NGS-CR and NGS-OM as alternative monitoring strategies that may reduce costs associated with monitoring kit fox populations, while providing precise and accurate estimates of population parameters (Waits and Paetkau 2005, Lukacs et al. 2009) and occupancy parameters, respectively. Furthermore, NGS techniques allow for the concurrent collection of samples from sympatric species (e.g., coyotes, red foxes, bobcats) without any additional effort or sampling costs.

A pilot study to assess the rate of kit fox scat deposition and fecal DNA degradation within each sampling season informed the sampling design, effectively balancing sample accumulation and degradation (see section 2.2). Genotyping errors were effectively minimized using procedures detailed in section 2.3.

*Field sampling*

Sampling occurred during primary periods annually that coincided with the periods preceding reproduction (January to March) and dispersal (July and August; see Section 5.4 for details), over a two year period. Within each primary period (session), we employed two spatio-temporal sampling designs. The first, employed a Pollock's robust sampling design, in which sampling occurred along 30 5 km transects (hereafter, multi-occasion transects) that were each surveyed 3–5 times (secondary sampling periods, or occasions) per session (i.e., temporal replication; Figure 18). The duration between sampling occasions was set to ~14 days based on the pilot study and allowed adequate time for scats to accumulate, limited the effect of DNA degradation, and minimized the violation of the closure assumption (see Section 2.2). Additionally, as part of a concurrent evaluation of canid occupancy patterns, 60 sites (each 6.25 $km^2$) were randomly selected without replacement from a grid of 576 cells superimposed on the study area and excluding cells containing any portion of a multi-occasion transect. Within each site, we established four 500 m transects (hereafter, single-occasion transects) along roadways (Figure 18) and surveyed each transect once per session.

Kit fox fecal samples were collected along transects in and around DPG (Figure 18). Two researchers surveyed each transect for carnivore scats. We recorded the location of each scat detected and collected ~0.7 mL of fecal material from the side of the scat (Stenglein et al.

2010a). Samples were preserved in 1.4 mL of DETs buffer (Seutin et al. 1991) and remaining portions of scats were removed. The GPS location of each scat was recorded, along with covariate data (i.e., habitat type, road type, position, scat measurement). Additional information was obtained from GIS layers for each scat's location, including soil composition and distance to water.

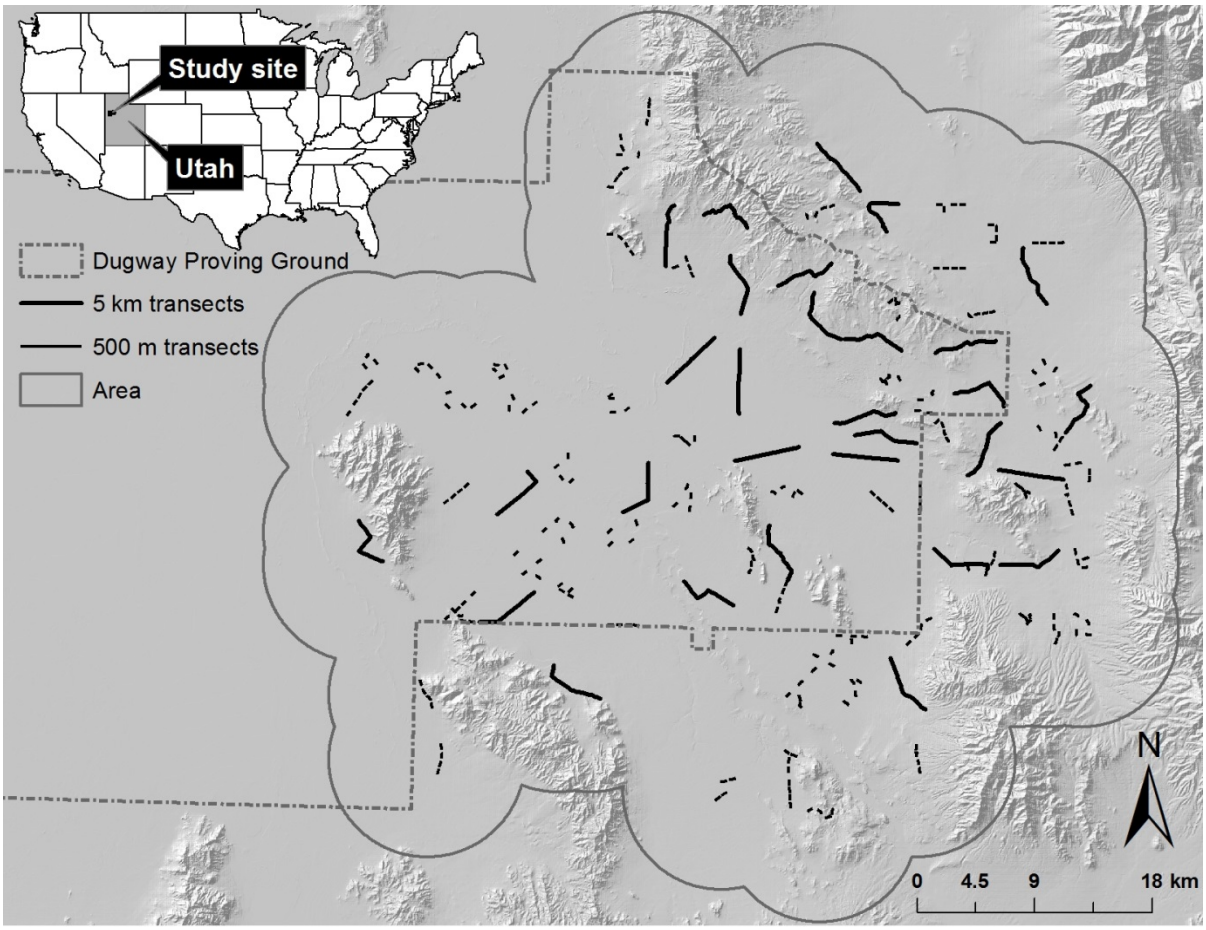



**Figure 18. Location of 5 km multi-occasion and 500 m single-occasion transects surveyed for kit fox scats, 2013–2014. Area boundary represents the effective sampling area used in spatially explicit capture-recapture models.**

Scat accumulation rates were greater in summer than winter (Section 2.2) and we surveyed multi-occasion transects four and three times during initial winter (2013) and summer (2013) sampling sessions, respectively (Table 11); we expected these levels of effort to yield a sufficient number of samples (Solberg et al. 2006). We subsequently performed power analyses to evaluate the number of occasions required to achieve a CV <10% for our abundance estimates when employing closed-capture analyses. For each analysis, 1,000 simulations were run in program MARK (White and Burnham 1999) using estimates of capture probability ($p$) generated from preliminary closed-capture models that considered temporal variation in $p$ and the number of individuals captured in each session. Across simulations, we assumed no behavioral response to sample collection and set recapture probabilities ($c$) equal to $p$. Power analyses indicated our

sampling effort was insufficient to achieve desired levels of precision for kit fox estimates, but that increasing our sampling to five winter and four summer occasions was sufficient; we increased our sampling in year 3 (2014) accordingly (Table 11).

**Table 11. Survey effort for kit fox (*Vulpes macrotis*) fecal DNA sampling and number of samples collected for estimating population abundance in western Utah, USA, over two winter (W) and two summer (S) sessions.**

| | Multi-occasion transects | | | Single-occasion transects | | | Carnivore Scats | |
|---|---|---|---|---|---|---|---|---|
| Session | Transects[a] | Surveys[b] | Total | Sites[c] | Surveys[b] | Total | Total | Kit fox[e] |
| W 2013 | 30 | 4 | 600 km | 60 | 1 | 120 km | 602 | 151 |
| S 2013 | 30 | 3 | 450 km | 60 | 1 | 120 km | 1,078 | 175 |
| W 2014 | 30 | 5 | 750 km | 60 | 1 | 120 km | 1,013 | 301 |
| S 2014 | 30 | 4 | 600 km | 60 | 1 | 120 km | 1,059 | 183 |
| | Temporal replication | | | Spatial Replication | | | 3,752 | 810 |

[a]Multi-occasion transects were 5 km in length
[b]Number of temporally replicated surveys conducted within a session
[c]Sites each contained four 500 m transects
[e]Number of scats determined to be kit fox based on genetic analyses (see Section 5.6 for details)

For subsequent NGS-CR analyses, we considered samples from multi-occasion and single-occasion transects when developing encounter histories. For NGS-OM analyses, we considered all samples collected on single-occasion transects located within the 60 randomly selected sites (Section 5.3). Furthermore, 43 additional sites already contained ≥2 km of the 30 multi-occasion transects; we delineated four 500 m (nested) segments within each of these sites to constitute spatial replicates. Thus for the NGS-OM analyses, we also considered those scats detected on the nested segments during the first sampling occasion within each session (to ensure equal effort with single-occasion transects). In total, NGS-CR analyses included samples from 30 5 km multi-occasion transects and 240 0.5 km single-occasion transects, while NGS-OM analyses included 103 sites (6.25 $km^2$), each containing four 500 m transects. Collectively, multi-occasion and single-occasion transects encompassed ~3,015 $km^2$, with an estimated effective sampling area (the area to which abundance estimates relate) of 3,663 $km^2$ ('Area' in Figure 18).

*Genetic laboratory analysis*

We initially intended to conduct species and individual identification (Section 5.3) on only those scats collected along the multi-occasion transects for NGS-CR analyses. In contrast, we intended to perform only species identification test on carnivore scats detected during single-occasion transect surveys, as only species is required for NGS-OM analyses. We obtained additional funding support from the National Geographic Society's Conservation Trust, which provided sufficient funding to conduct both species and individual identification on all samples collected

(i.e., samples from both multi-occasion and single-occasion transects), allowing abundance estimates and occupancy estimates to be obtained for the same spatial extent.

We performed the species identification test on all samples collected. Samples that failed to amply for mtDNA were repeated once to minimize sporadic effects (Murphy et al. 2007, Lonsinger et al. 2015b). The species identification served as an initial filter, allowing us cull low quality samples prior to individual identification (Section 2.3). For each sample retained, we then performed two replicates of individual identification and evaluated amplification success rates with ConGenR (Lonsinger and Waits 2015). Low quality samples failing at >50% of nDNA loci across the first two replicates were culled (Paetkau 2003; Section 2.3). For the remaining samples, we performed additional PCR replicates in sets of two until we established consensus genotypes at a sufficient number of loci (see Section 2.3) or reached a total of eight replicates. Genotyping errors were calculated following Broquet and Petit (2004) using the package ConGenR (Lonsinger and Waits 2015). As genotyping errors can lead to inflated population estimates (Waits and Leberg 2000, Lukacs et al. 2009), we scrutinized samples with identical or near identical multilocus genotypes, re-evaluating scoring of near matches to check for inconsistencies (Creel et al. 2003) and considering consistency in both sex identification and spatial attributes (Smith et al. 2006)

*Abundance and survival estimation*

For kit foxes, capture-recapture data were analyzed using maximum likelihood methods applied in (i) non-spatial Huggins closed-capture models (Huggins 1989), (ii) spatially-explicit capture-recapture (SECR) models (Borchers and Efford 2008) and (iii) CAPWIRE models (Miller et al. 2005). Both the Huggins closed-capture models and SECR models were fit using the entire NGS data set (i.e., all samples resulting in individual identification). For non-spatial models, multiple captures of an individual within an occasion were collapsed into a binary response to construct individual encounter histories. For SECR models, we followed procedures of Thompson et al. (2012) and Russell et al. (2012), and gridded the study area into cells, using the center of each cell as a 'conceptual' trap. We selected a grid size of 6.25 $km^2$ (2.5 x 2.5 km), by considering the home range size and movement capacity of kit foxes (Koopman et al. 2000, Cypher and List 2003, Dempsey et al. 2015). The grid aligned with that used to identify single-occasion sites and each of these sites represented one conceptual trap. Additionally, the grid bisected longer, multi-occasion transects, demarcating multiple traps from each transect. Captures of an individual across multiple traps within a single occasion can be used by SECR to characterize the spatial point process (Borchers and Efford 2008). Consequently, capture histories included all captures and we assigned each detected scat to the location of a conceptual trap. As a result, scats of a single individual detected within a grid cell during a single occasion were assigned to the same location, effectively treating clusters of scats as single observations and reducing the influence of spatial autocorrelation on density estimates (Thompson et al. 2012).

Effort varied across transects and grid cells. To account for variation in effort between single-occasion and multi-occasion sites in Huggins capture-recapture models, we distinguished males and females captured on multi-occasion transects from those captured only on single-occasion transects (i.e., multi-occasion males, multi-occasion females, single-occasion males, single-occasion females), and for each sex applied the mean *p* estimated from multi-occasion transects to single-occasion transects. For SECR models, effort related to each trap and we used the total

length of transects surveyed within each grid cell to represent effort (where repeat surveys on multi-occasion transects were summed).

CAPWIRE assumes equal effort across sites (Miller et al. 2005). To compare the performance of CAPWIRE with multi-session models, we fit separate CAPWIRE models for each session, using a reduced data set that met the equal effort assumption and was intended to represent how managers would sample if using this estimator (single-occasion formulation). Specifically, we identified portions of the multi-occasion transects contained within one of the 576 grid cells used to select single-occasion transects, and that were ≥2 km in length, allowing four 500 m nested transects to be identified. For each session, we then considered captures from single-occasions transects and only the first occasion of multi-occasion transects, restricting captures to those on nested transects when estimating abundance. Initial CAPWIRE abundance estimates for both species were generally lower across sessions than those generated with multi-session models (see Section 5.6). To determine if CAPWIRE would produce estimates more comparable to the multi-session models with a more complete dataset, we increased the number of captures included in the analysis by including captures from all occasions and dividing the number of captures by the number of occasions to standardize effort (multi-occasion formulation).

Non-spatial Huggins closed-capture models were fit using a robust design framework (Huggins 1989, Pollock et al. 1990) in program MARK (White and Burnham 1999). We tested for closure within each session with CLOSETEST (Stanley and Burnham 1999). This modelling framework provides estimates of apparent survival (*S*), *p*, and *c*, as well as inferences regarding temporary immigration ($1 - \gamma''$) and temporary emigration ($\gamma'$). We describe the model set an report estimates for related to *S*, *p* and derived estimates of abundance. We compare estimates of *S* to those obtained via radio-telemetered kit foxes (provided by Drs. Eric Gese and Bryan Kluever, USU). Recapture was always set to equal *p*, We compare estimates of abundance with those resulting from alternative analyses (i.e., SECR and CAPWIRE analyses).

We modeled apparent survival considering models with constant, time-varying, or trend in survival (Otis et al. 1978, Williams et al.2002). We also considered models in which survival varied by season (winter-summer vs. summer-winter) or was influenced by an extreme winter (winter 2013). We considered the effects of sex, individual heterozygosity (i.e., individual genetic diversity), and distance to nearest water on survival. Additionally, evidence from our study site indicates that intraguild predation by coyotes can account for a significant proportion of kit fox mortalities (Arjo et al. 2007, Kluever 2015). Thus we also considered models for apparent survival that incorporated a covariate indexing coyote activity. We considered three movement models which describe how animals move in and out of the study area between sessions: random movement ($\gamma' = \gamma''$), constant but different γ parameters ($\gamma' \neq \gamma''$), and no movement ($\gamma' = \gamma'' = 0$). We did not expect a behavioral response to capture when using NGS and as previously described, set $p = c$. We modeled *p* as constant or varying by time, trend, and sex within sessions, and considering additive models of sex with both time and trend. We combined each model for *S*, with each combination of models for movement and *p*. For each species, we used AICc) and Akaike weights to compare the relative fit of models (Burnham and Anderson 2002). Parameter estimates accounting for model-selection uncertainty were achieved by model-averaging (Burnham and Anderson 2002). We calculated variances and confidence intervals for model-averaged estimates with the delta method (Williams et al. 2002).

We fit SECR models by maximizing the full likelihood with the R package 'secr' (Efford 2015; R Core Team 2015), and using a multi-session formulation, which allowed improved estimation of parameters shared across sessions (Efford et al. 2009). In addition to estimating density (*D*), SECR models estimate *g0* and $\sigma$, which combined replace *p* and jointly describe the decline in detectability with increasing distance between the trapping array and an animal's activity center (Efford et al. 2009). We utilized a half-normal detection function (or circular bivariate normal home range), in which *g0* and $\sigma$ represent the intercept and scale parameter, respectively (Efford et al. 2009).

In order to estimate density (and derive abundance = *D* * effective sampling area), the effective sampling area (i.e., the state space) must be appropriately defined. We evaluated the effect of buffer width around traps by considering changes in the log-likelihood, *D*, and the effective sampling area, while increasing widths from 1–15 km. We selected the width where the rate of change in both the log-likelihood and effective sampling area stabilized, and where *D* stabilized at the fifth decimal place. We applied this buffer around traps, creating a habitat mask with grid points evenly distributed every 2.5 km; spatial covariates characterizing the area around each point (within a 1.25 km radius)—the majority soil type and habitat, the proportion of shrubland and woodland cover, and the mean distance to water—were extracted for modeling spatial variation in *D*. We first evaluated capture models in which *g0* and $\sigma$ varied across sessions and was either constant or varied by time, trend, or sex within sessions. Additionally, we considered both interaction and additive effect models of sex with time and trend. We then used the best-fit capture model (i.e., the model with the lowest AICc), when fitting models for *D*. Models formulated for *D* allowed variation among sessions. Additionally, we fit models of *D* (overall and by sex) using the aforementioned spatial covariates believed to influence kit fox space-use, and combinations of these predictors. We used AICc and Akaike weights (Burnham and Anderson 2002) to compare the relative fit of models for each species. Single top models of capture and *D* could be identified, with the next closest model having little to no support based on $\Delta$AICc. We calculated abundance and confidence bounds by multiplying session specific $\hat{D}$ and associated confidence limits by the effective sampling area (Russell et al. 2012).

For CAPWIRE models, we fit each session independently with the R package 'capwire' (Pennell et al. 2013, R Core Team 2015). CAPWIRE models assume either that all individuals have equal *p* (equal capture model; ECM) or that two capture classes exist (two-innate rates model; TIRM) representing individuals with relatively low and high *p* (Miller et al. 2005). We fit both single-occasion and multi-occasion formulations of the ECM and TIRM for each session, and compared model fit using a likelihood-ratio test implemented in 'capwire' with 1,000 simulations; the ECM was rejected when $P < 0.1$. We subsequently generated 95% confidence intervals for the estimate of the best supported model using 1,000 parametric bootstraps (Miller et al. 2005, Pennell et al. 2013).

*Occupancy, space-use, and dynamic processes*

As with NGS-CR analyses, we refer to each sampling season, over which occupancy was assumed to be constant, as a 'session'. To describe sampling and results, we refer to each randomly selected site, or patch, as a 'site', and each spatial replicate (i.e., each survey) within a site as a 'transect'. We identified a desired site size of 6.25 $km^2$ (2.5 x 2.5 km), an area similar to the average home ranges reported for kit foxes (2.5–11.6 $km^2$; Cypher and List 2003). We conducted four surveys across 103 sites per session; to maximize spatial coverage and minimize

field costs, we used spatial replication by establishing four 500 m transects within each randomly selected site (Section 5.5.1, Figure 18). Although sampling spatial replicates without replacement can bias parameter estimates (Kendall and White 2009), sampling with replacement may be impractical for NGS when all surveys are conducted within a single site visit and searcher efficiency is high (i.e., all or most of the scats present are detected). Alternatively, sampling spatial replicates without replacement does not bias results if occupancy is constant for each transect (Guillera-Arroita 2011) or the target species is highly mobile (Kendall and White 2009, Harris et al. 2014).

Those NGS surveys contributing to occupancy modeling analyses were conducted during two winter (14 January to 6 March 2013; 13 January to 19 March 2014) and two summer (12 July to 16 August 2013; 10 July to 21 August 2014) sessions. Each site was visited once per session during which each transect was surveyed following procedures detailed in Section 5.5.1. We performed a species identification test on all samples collected following procedures detailed in Sections 2.3 and 5.5.1).

Covariates used to model variation in occupancy parameters were obtained from available GIS layers. We processed all GIS layers with ArcGIS 10. We expected soil to influence kit foxes, as they utilize burrows year-round (Arjo et al. 2003, Kozlowski et al. 2008); soil layers were obtained from the Utah Automated Geographic Reference Center (http://gis.utah.gov/), and we reclassified soil types into four categories (silt, fine sand, blocky loam, and gravel; *sensu* Dempsey et al. 2015). Data on prey densities and diversity were not available across sites, but land cover influences prey abundance and diversity at our study site. Shrubland and woodland habitats (i.e., shrub-steppe, greasewood, vegetated dunes, and open juniper woodland) at DPG supported higher prey diversity and abundance than grasslands, and chenopod, pickleweed, and urban habitats supported the lowest prey resources (Arjo et al. 2007, Kozlowski et al. 2008, 2012). We utilized 2012 LANDFIRE (http://landfire.cr.usgs.gov/) vegetation layers to calculate the proportion of shrubland and woodland habitat (%SW) in each site, presumably representing relatively prey-rich habitats (Kozlowski et al. 2012) and greater thermal cover (Blaum et al. 2007) for larger bodied intraguild predators (i.e., coyotes). Water availability was predicted to influence canid space use (Arjo et al. 2007, Hall et al. 2013). Perennial water sources were identified by the DPG Natural Resource Program GIS layers. We utilized Google Earth imagery to locate additional (unmapped) water sources by following livestock and horse trails to convergence points and ground-truthing points to confirm the presence of water. For each site, we characterized water in three ways: (i) distance to nearest water, and the number of water sources within (ii) 2.5 km and (iii) 5 km from the site center. Road density may influence the canid detection or occupancy. We obtained road layers from the Utah Automated Geographic Reference Center and calculated road density for each site.

We collected additional covariates during field surveys. Road characteristics can influence scat persistence (Lonsinger et al. 2016) and detection (Kluever et al. 2015). During each survey, we characterized the transect's road type as (1) an unmaintained two-track road, or a maintained (2) single-lane or (3) two-lane gravel road (*sensu* Lonsinger et al. 2016). Detection of scats may be influenced by snow cover, survey date, and/or survey time (Harris et al. 2014); we recorded these covariates during each survey. Snow can reduce detection by covering scats (see Section 2.3). Date may further influence detection, if canid activity changes throughout winter (e.g., during reproduction) or summer (e.g., increased juvenile activity). The time of surveys was used to

characterize the angle of the sun, which may influence visibility and shadowing effects, and was standardized across seasons as time from solar noon. Finally, to evaluate the influence of coyotes on kit fox occupancy and dynamics, we characterized coyote activity at the site and transect levels. At the site level, we characterized coyote activity as (1) the total number of coyote scats detected, and (2) the total number of transects on which coyotes were detected. At the transect (i.e., survey) level, we characterized coyote activity as (1) the number scats detected, and (2) the detection or non-detection of coyotes.

We assumed kit fox occurrence did not influence coyote space use, as mammalian intraguild predation is typically unidirectional and size-mediated (Verdy and Amarasekare 2010, Lourenço et al. 2013). We employed a multi-stage approach using program MARK (White and Burnham 1999). For each stage, we used AICc to compare the relative fit of models and cumulative Akaike weights to evaluate predictor importance (Burnham and Anderson 2002). We initially considered using dynamic co-occurrence models to evaluate the influence of coyotes on kit fox occupancy (Richmond et al. 2010). Thus, we first used dynamic single-species occupancy models (MacKenzie et al. 2003) to estimate coyote occupancy; coyote occupancy was very high (not significantly different from 1 across sessions; Lonsinger 2015), effectively eliminating our ability to evaluate patterns of co-occurrence at the site level (Richmond et al. 2010). Instead, we used dynamic single-species occupancy models for kit foxes that included both environmental covariates and indices of coyote activity, exploiting the variation in coyote activity at the site and transect levels to explore the influence of coyotes on kit fox spatial dynamics at multiple scales. Under this framework, we interpreted variation in kit fox *p* among transects of an occupied site as reflecting differences in fine-scale space use (i.e., a behavioral response).

We evaluated correlations among covariates with a Kendall's rank correlation test. Only the three characterizations of water were correlated with one another ($r > |.48|$, $P < 0.001$) and we never included >1 water variable in a given model. We used a structured modeling approach, first identifying the best global model and then sequentially fitting models for probability of detection (*p*), proportion of area occupied (or occupancy; ψ), and the dynamic parameters (local extinction [ε] and colonization [γ]) together. We considered global models for ψ, ε, and γ that contained %SW, soil, site-level coyote activity, road density, and water availability (and time variation for ε and γ); site-level coyote activity in models for ε and γ reflected coyote activity in the preceding session. We considered global models for *p* containing transect-level coyote activity, road type, road density, presence of snow, date, sun (i.e., difference between survey time and solar noon), and variation among sessions.

Four covariates had >1 characterization: road type (ordinal vs. categorical), water availability (distance to nearest vs. sources within 2.5 or 5 km), site-level coyote activity (number of scats vs. number of transects), and transect-level coyote activity (number of scats vs. detection/non-detection). To identify the best global model, we first compared the fit of models containing all possible combinations of each of these four covariates and retained the most supported characterizations of each predictor for subsequent analyses.

After identifying the best-fit global model, we fit all possible combinations of predictors for *p*, while maintaining the global models for ψ, ε, and γ, to identify the best detection model. Next, using the best-fit model for *p*, and the global models for ε and γ, we fit all possible combinations of predictors for ψ and identified the model with the lowest AICc. Finally, we used the best-fit models for *p* and ψ and simultaneously evaluated models for the dynamic parameters,

considering all possible combinations of predictors for ε and γ both within and across parameters.

### 5.5.2 Barry M. Goldwater Range and Cabeza Prieta NWR, Arizona

Sonoran pronghorn are distributed over approximately 11,000 $km^2$ (USFWS 2010), but low population density makes detection on the periphery of their range difficult. To increase efficiency, we implemented a targeted sampling approach (Puechmaille and Petit 2007, Rudnick et al. 2008, Stenglein et al. 2010b). Radio-telemetry and aerial surveys have shown that Sonoran pronghorn use areas close to natural and developed water sources more often than random locations (deVos and Miller 2005, Morgart et al. 2005) making targeted sampling at drinkers a practical method for sampling a majority of the population. In May and June 2013 and 2014, we attempted to collect fecal samples three times (six total) at an interval of seven days at all developed drinkers likely to be used by pronghorn (17) (Table 12, Figure 19) to maximize the chance of obtaining usable DNA (Woodruff et al. 2014, 2015). However, actual sampling locations, frequencies, and intervals were limited by logistic constraints on agency access, minimizing pronghorn disturbance, terrain, and level of pronghorn use. Because of these limitations, we classified each sampling site into one of two session types: single-session and multi-session. Based on relocations of >100 radio-collared pronghorn from 2006–2013, approximately 30% of the population never visited a drinker during summer (J. Hervert, personal communication) but the true level of drinker visitation is unknown. Therefore in 2014, we targeted groups with radio-collared individuals located away from (>1 km) drinkers and opportunistically collected samples from 9 locations after observing defecation events (Fig. 1). Based on 2013 sampling results and weather conditions (i.e., wetter conditions and better forage), we started 2014 sampling 12 days later than in 2013.

Samples were collected from the area within 50 m of drinkers (Woodruff et al. 2015), and we excluded piles that appeared to be from >1 individual based on pellet shape, color, and size, as these were likely mixed samples. At multi-session sites the collection area was divided into four quadrants (Q1–Q4) for later subsampling (see below). For each sampling event, we attempted to collect samples at a rate of three times the number of pronghorn counted at the drinker just prior to sampling (based on direct observation and motion-sensing cameras). We chose this target number of samples to ensure sufficient recaptures/redetections and maximize performance of capture-recapture estimators (Solberg et al. 2006). At single-session drinkers, we sampled from all acceptable pellet piles. At least six pellets were collected from each sample, placed in paper coin envelopes, and stored at room temperature in a plastic Ziploc bag with (~250 ml) of silica desiccant (Fisher catalogue no. S161-212) to reduce DNA degradation. All remaining pellet groups were crushed or scattered to avoid resampling when searching the same area during a later sampling period, and thus, we assumed samples collected in the next occasion were deposited during the interim period. We classified samples by freshness (F1: Freshest; visibly wet on outside of pellet; F2: Less fresh; wet/moist on inside of pellet only, crushes easily; F3: Oldest; no moisture, crumbles when crushed) for later subsampling. We used a chi-square analysis test of independence (R version 3.1.2, www.r-project.org) to assess individual ID success by freshness and session (1–3). Samples were also field-classified based on visual inspection of size and morphology as adult (≥1 year old) or fawn (<1 year old). For 76% of individuals we cross-checked our age assignment against a fitted cross-validation model

developed from multiple pellet measurements (see Section 2.2) of known age individuals (Woodruff et al. 2016a). We were not able to cross check the other 24% of samples due to having no remaining pellets post-extraction.

*Genetic Laboratory Analysis*

We analyzed all samples from single-session sites and approximately 2 times the number of individuals estimated to be using the drinker from multi-session sites starting with the freshest samples. Genotyping errors were calculated following Broquet and Petit (2004) using the package ConGenR (Lonsinger and Waits 2015) in R (R Core Team 2015). As genotyping errors can lead to inflated population estimates (Waits and Leberg 2000, Lukacs et al. 2009), we reanalyzed samples mismatching at 1 or 2 loci following methods similar to Kendall et al. (2009). Samples with continuing ambiguity were amplified in a second multiplex with an additional 6 loci to refute or confirm a match.

**Table 12. Sampling design and dates for Sonoran pronghorn fecal pellet collection in Year 2 (2013) and Year 3 (2014).**

| | 2013 | | | 2014 | | |
|---|---|---|---|---|---|---|
| **Sampling Location*** | **Sess. 1** | **Sess. 2** | **Sess. 3** | **Sess. 4** | **Sess. 5** | **Sess. 6** |
| East Release (ER), Morgart (MG) | May 25 | May 31 | June 5 | June 9 | June 16 | June 23 |
| Charlie Bell (CB) | May 31 | June 6 | June 11 | June 6 | June 13 | June 20 |
| Point of Pintas (PP), Devil Hills (DH) | June 3 | June 10 | June 17 | June 3 | June 12 | June 18 |
| Uken (UK), New Halliwill (NH) | May 31[a] | June 8 | June 15[b] | June 15 | June 22 | June 28 |
| Little Tule (LT) | May 31 | June 6 | | June 7 | June 20 | |
| Adobe Well (AW), Adobe Forage Plot (AFP), Lower Well (LW) | May 30 | | | June 14 | | |
| Granite Mountain (GM) | June 5 | | | June 10 | | |
| Sierra Pintas (SP) 1, 2, 3 | June 5 | | | June 10 | | |
| Fawn Hills (FH) | June 11 | | | June 24 | | |
| Antelope Parabolic (AP) | June 11 | | | June 20 | | |
| 3 Jack (3J) | | | | June 8 | June 15 | June 28 |

[a]UK only. NH sampled only once in 2013 due to access limitations on Barry M. Goldwater Range

*Subsampling method*

At multi-session sites the collection area was divided into 4 quadrants (Q1–Q4) for later subsampling. At single-session drinkers, we sampled from all fresh pellet piles which appeared to be from >1 individual based on pellet shape, color, and size. We initially analyzed a subsample of approximately two times the number of individuals estimated to be using the drinker from multi-session sites and all samples from single-session sites. We first analyzed the freshest samples (F1s) (Lucchini et al. 2002) and then F2s. If we had too many F2s, we subsampled equally across quadrants choosing every other numbered sample. F3 samples were used only if necessary to reach target sample size and were subsampled across quadrants in the same manner.

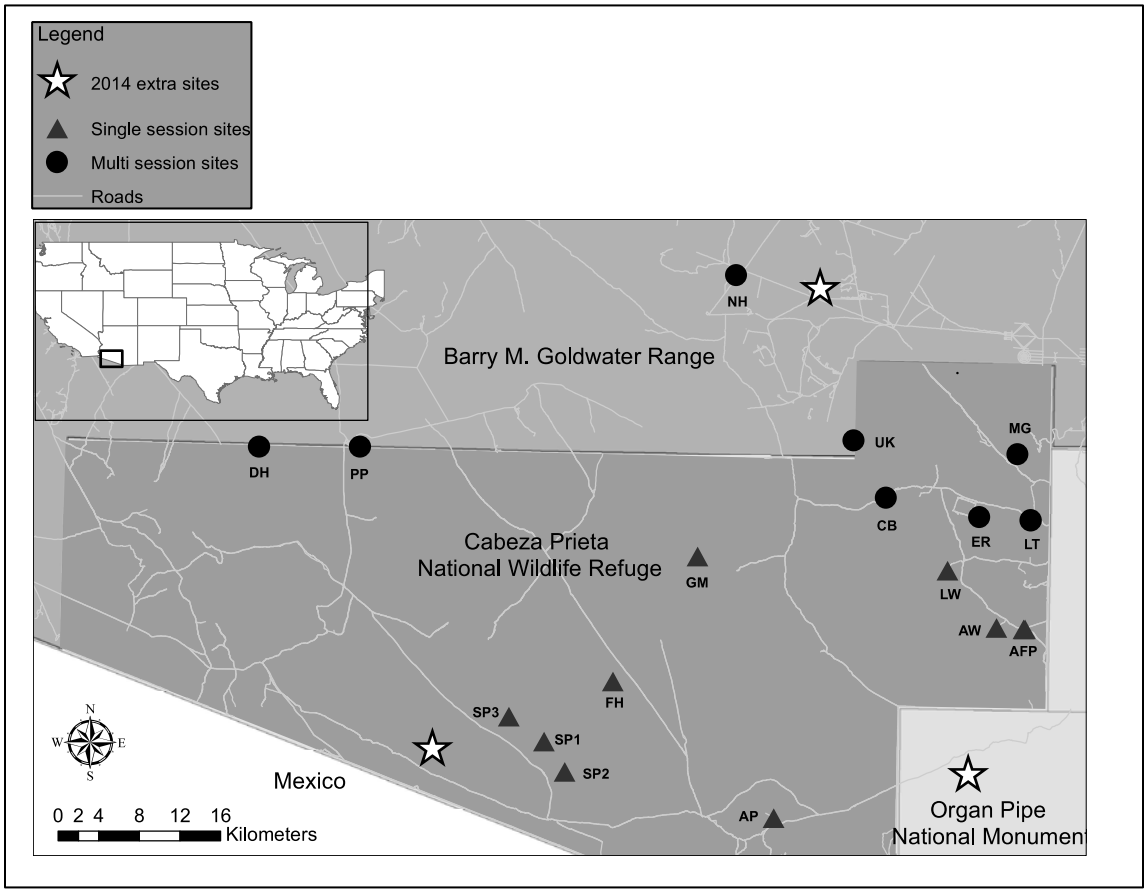



**Figure 19. Study area and location of sampling sites on Barry M. Goldwater Range and Cabeza Prieta NWR for noninvasive genetic sampling of Sonoran Pronghorn.**

Following the analysis of year 2 samples, we performed a power analysis to inform year 3 sampling and to determine the level of effort required for long-term monitoring of the Sonoran pronghorn population. Our power analysis indicated our sampling protocol was sufficient, and thus, the procedure for sample collection and analyses remained the same in both years.

*Abundance and Survival Estimation*

Each successfully amplified sample was considered a detection. We generated an encounter history for each individual indicating detected (1) or not (0) in each sampling session, counting only a single detection per individual per sampling session. We collapsed sessions 3 and 4 into a single session since only 3 sites were sampled 4 times.

*Adult*

We applied Huggins' robust design in the development of 4 biologically appropriate models of survival and abundance (see Table 25 in section 5.6) which allowed varying survival and detection probabilities with time and variation in capture and recapture probability, (Pollock 1982, Pollock et al. 1990, Huggins 1989) in Program MARK (White and Burnham 1999). We used Huggins' models because our models included an individual covariate, and we believe we sampled a substantial proportion of the population. The population was assumed to be demographically and geographically closed within the annual sampling occasions during which we estimated capture/detection ($p$) and recapture/redetection ($c$) probabilities, and abundance ($N$). Across years, gains (birth and immigration) and losses (death and emigration) in the population are expected and we estimated annual apparent survival ($\varphi$). For survival, we modeled equal survival probability for male and female adults as well as varying survival probabilities by sex. Additionally, we modeled the individual covariate fed/not fed on survival probability because supplemental feed is not provided at all sites.

Data from single and multi-session sites were combined in the same model. While redetection of individuals from single-session drinkers was possible at multi-session drinkers and vice versa, we never detected the same individual at both single and multi-session drinkers within the same year. We hypothesized that drinker visitation could differ by sex and estimated detection and redetection probabilities and abundance in groups (males and females at both single-session and multi-session sites) resulting in 4 estimates. To evaluate differences between sessions in a single year, we modeled time varying detection and redetection probabilities. To estimate detection probability at single-session sites, we used the model-estimated mean detection probability from multi-session sites by sex (i.e., mean of males' detection probability at multi-session sites equals detection probability for males at single-session sites). Mean detection probability by sex was estimated across all of the data using the means coding in the design matrix. Before fitting models, parameter estimates for single-session site redetection probabilities were fixed at zero and emigration parameters ($\delta$) were also fixed to zero due to lack of precision (Lukacs et al. 2009). Additionally, because we expanded our sampling extent in 2014, we performed analyses on drinker locations only (i.e., no individuals from non-drinker sites) from 2013 and 2014, as well as on all locations.

*Fawn*

We used separate closed capture models in each year to estimate fawn detection and redetection probabilities and abundance in 2013 and 2014, again, modeling drinker only and all locations separately. We used a full likelihood robust design model to estimate annual apparent survival for the 2013 fawn cohort. We included models with and without time varying detection and redetection probabilities. We estimated fawn survival over the interval from approximately 3 months to 15 months of age, largely outside the highly vulnerable neonatal period (i.e., < 1 month of age). Survival in neonates is highly stochastic, and analysis of survival in older fawns

that have survived the neonatal period may be more sensitive to current environmental conditions or management practices and provide a more meaningful indicator of current population growth.

All adult and fawn models assumed equal detection and redetection probability (i.e., no behavioral effect), as it is unlikely that the initial detection would affect subsequent detections. We did not detect fawns at single-session sites in 2013; however, in 2014 we did and used the model-estimated mean fawn detection probability at multi-session sites for single-session sites. We used $AIC_c$ corrected for small sample size to evaluate relative support for each model. We model averaged parameter estimates and standard errors over all models (Burnham & Anderson 2002). We summed population estimates for each group (6 groups: single and multi-session for each of adult male, adult female, and fawn) and calculated standard errors for abundance using the Delta method (Seber 1982).

*Comparing abundance estimators and simulations*

In order to evaluate levels of precision under reduced sampling efforts and the use of alternative abundance estimators, we also analyzed our data (1) with a reduction in number of secondary periods per primary period and the number of samples analyzed per secondary period; (2) by reanalyzing the data using single-session (*capwire*) models; and (3) using simulations of closed populations to estimate the optimal number of consensus genotypes needed for precise abundance estimates (CV≤10–20%; Pollock et al.1990).

As part of our monitoring research (Woodruff 2015, Woodruff et al., 2016b), we extracted and genotyped 494 and 692 fecal samples in 2013 and 2014, respectively (reported in 5.6.2). In 2013, we later included an additional 138 samples (hereafter, extra samples) from sessions 2 and 3 to investigate changes in abundance estimates and precision with a larger sample size. DNA analysis resulted in 474 and 476 captures of 91 and 110 individuals in 2013 and 2014, respectively. Seventy-five captures and three individuals were from extra samples in 2013. Capture-recapture analyses were performed using a CMR model (Otis et al. 1978) in Program MARK. CV was ~2% in both years indicating the potential to save time and money by reducing sampling effort (e.g., fewer sessions, fewer samples) while still producing reliable population estimates.

For simulations, we simulated encounter histories for true abundance of 100–300 individuals, a sample size of 0.33–3.33 samples/individual/session, in 1–3 sampling sessions. We explored trade-offs between sample size, number of sessions, and multi-session (CMR) versus single-session (*capwire*) closed capture-recapture abundance estimators, and the need for an accurate and precise estimate. In multi-session models, populations are sampled during multiple sessions at distinct time points or within discrete sampling sessions (Chao 2001). Although there may be multiple captures of the same individual within a sampling session, only a single capture per individual per sampling session is counted. In contrast, *capwire* uses the total number of captures for each individual and allows capture of an individual multiple times within sessions (Miller et al. 2005, Pennell et al. 2013).

We evaluated the performance of each sampling design and estimator by comparing the simulated abundance estimates to the true population size (percent bias), the CV, and the relative mean squared error (RMSE). The CV is commonly used to describe the precision of an abundance estimate. As a general rule, CV <10% is ideal, however, <20% indicates a precise

estimate (White et al. 1982). CV does not, however, measure model fit, as CV could be very small with a poorly fit model. RMSE incorporates accuracy (bias) and precision (variance) and low values indicate a good balance between bias and precision. When RMSE values are >0.5, CI coverage is generally poor. Additionally we examined the 95% confidence interval (CI) coverage described as the proportion of times (out of 100 in this case) the true value was contained within the interval.

## 5.6 SAMPLING RESULTS

### 5.6.1 Dugway Proving Ground, Utah

*NGS-CR sampling and species identification*

Within each session, 570–870 km of transects were surveyed (combined multi-occasion and single-occasion transects, Table 11). The mean time between multi-occasion transect surveys (within a session) was ~14 days (mean = 13.7 ± 0.93 SD, range = 9–18), with departures from 14 days resulting from access constraints (e.g., heavy snow fall, military training activities). We collected 3,752 carnivore scats (Table 11). We observed high mtDNA amplification rates, with successful species identification for 93.3% of winter and 82.8% of summer samples. We identified 21.6% and 63.3% of samples as kit fox and coyote, respectively (Table 13). Only 2.5% of samples were from other non-target carnivores, 1.1% were mixed (i.e., contained DNA of >1 species), and 11.5% failed species identification tests (Table 13).

Winter power analyses indicated four occasions failed to achieve a CV <10% for kit foxes. Observed *p* increased across occasions as snow melted (Table 14); nearly all snow had melted by the final occasion and we assumed that *p* of a fifth occasion would have been comparable to the fourth, so we set them equal to one another while conducting a power analysis for five occasions. Five winter occasions produced a CV = 6.5%. Observed kit fox *p* was relatively stable across summer occasions (Table 14). Three occasions were insufficient to achieve the desired level of precision. We again assumed that the *p* of a final occasion would be comparable to that observed during the subsequent occasion and set them equal to one another. Simulation indicated that four occasions in summer would produce a CV = 9.7%. Consequently, we increased sampling effort in 2014 to five winter and four summer occasions (Table 11).

*Individual identification*

The average number of alleles per locus was 8.1 (range = 5–14; SD = 3.07) and mean expected heterozygosity of the 9 loci was 0.64 (range = 0.46–0.77; SD = 1.10). Six loci were required to achieve a P(ID)sibs <0.01 for kit foxes, excluding sex identification markers. Kit fox individual identification success rates (i.e., the proportion of samples identified to species for which a successful individual identification was achieved) ranged from 59.4% (summer 2013) to 91.4% (winter 2013). Across sessions, 109 kit foxes were identified (Table 13), among which 102 individuals had consensus genotypes at ≥8 loci. Sex was determined for all individuals. We captured 36–50 kit foxes each session (Table 13) and 37 individuals across >1 sessions. We captured more males (60%) than females (Table 13). For samples in the final dataset, genotyping

error rates were low (overall allelic dropout rate = 17.3%; overall false allele rate = 3.4%), suggesting the probability of a genotyping error with the mean number of replicates (5) was low (i.e., $[0.1733 + 0.0342]^5 = 3.85 \times 10^{-4}$).

**Table 13. Number of scats detected during fecal DNA surveys identified as kit fox (*Vulpes macrotis*), coyote (*Canis latrans*), or another non-target carnivore (NTC; domestic dog, red fox [*V. vulpes*], bobcat [*Lynx rufus*], and cougar [*Puma concolor*]) based on mitochondrial DNA species identification. Minimum number known alive (MNKA) and proportion male (M) for kit foxes indicates the number of unique genotypes detected. Total MKNA is the number of unique individuals identified throughout the study. Mixed samples contained mitochondrial DNA from >1 species. Samples were collected in over two winter (W) and two summer (S) sessions in western Utah, USA, 2013–2014.**

| | Kit fox | | Coyote | Other | | | |
|---|---|---|---|---|---|---|---|
| Session | Scats | MNKA (M) | Scats | NTC | Mixed | Failed | Total |
| W 2013 | 151 | 40 (0.68) | 378 | 9 | 3 | 61 | 602 |
| S 2013 | 175 | 36 (0.56) | 626 | 37 | 10 | 230 | 1,078 |
| W 2014 | 301 | 50 (0.58) | 645 | 23 | 16 | 28 | 1,013 |
| S 2014 | 183 | 38 (0.47) | 725 | 23 | 15 | 113 | 1,059 |
| Total | 810 | 109 (0.60) | 2,374 | 92 | 44 | 432 | 3,752 |

*Robust design non-spatial capture-recapture analysis*

Program CLOSETEST supported the population closure assumption for kit foxes in 2013 (winter: $\chi^2 = 3.43$, df = 3, $P = 0.329$; summer: $\chi^2 = 1.19$, df = 2, $P = 0.550$), but not for 2014 (winter: $\chi^2 = 17.08$, df = 4, $P = 0.002$; summer: $\chi^2 = 8.38$, df = 3, $P = 0.006$). Component and subcomponent tests suggested closure violations may have resulted from population losses following the second occasion in both 2014 sessions.

We compared the fit of 36 non-spatial models for kit fox *S* (Appendix 5.2 in Lonsinger 2015). When fit with each combination of the six detection and three movement models (Appendix 5.2), each apparent survival model was represented 18 times in initial model sets. We excluded models for which *S* or *p* were confounded, or where boundary effects resulted in estimates of *S* or *p* fixed at 1 (SE = 0). Multiple models among the most supported shared similar structures for *S*, but differed in structure for *p* and movement (Appendix 5.3 in Lonsinger 2015).

Male kit fox survival ($S_M$) was slightly lower than female survival ($S_F$) across intervals and overall. Model-averaged kit fox survival was high in the period between winter 2013 and summer 2013 ($S_M = 0.82$, 95% CI = 0.26–0.98; $S_F = 0.87$, 95% CI = 0.28–0.99), high between summer 2013 and winter 2014 ($S_M = 0.81$, 95% CI = 0.19–0.98; $S_F = 0.87$, 95% CI = 0.24–0.99), and lower in the interval from winter 2014 to summer 2014 ($S_M = 0.59$, 95% CI = 0.11–0.94; $S_F$

= 0.67, 95% CI = 0.16–0.96). Winter 2013 experienced atypical snowfall amounts and may have increased survival during the following spring (i.e., due to increased moisture and primary productivity). Thus, we report annual survival rates for kit foxes from summer 2013 to summer 2014. During this 12 month period, $S_M$ and $S_F$ were 0.48 and 0.58, respectively. As is evidenced by our large confidence intervals for seasonal apparent survival, estimates had poor precision. Still, these results are similar to annual survival estimates generated from telemetered foxes and known fate models in our study region, which were 0.56 for adults and 0.29 for juveniles (Bryan Kluever, personal communication). We were unable to distinguish juveniles from adults based on NGS, and our results therefore do not distinguish among age classes.

Model-averaged estimates of kit fox *p* were similar between sexes and ranged from 0.186–0.536 in winter and 0.276–0.432 in summer (Table 14). The best-fit models suggested a trend in *p* within sessions (Table 14, Appendix 5.3 in Lonsinger 2015). The model-averaged abundance estimates from robust design non-spatial models suggested that there were 60.1–73.2 kit foxes present in the study area (Figure 20). The 95% confidence intervals associated with estimates suggested population abundance was similar across sessions (Figure 20). More traditional live-capture-recapture techniques, combined with radio-telemetry and den monitoring, which have historically been employed at DPG to monitor kit foxes populations, failed to have a sufficient number of initial captures and recaptures (i.e., insufficient sample sizes) to generate estimates of abundance (B. Kluever, personal communication).

*Multi-session spatially-explicit capture-recapture analysis*

Transects were distributed within 146 grid cells and mean spacing between these conceptual traps was 2.7 km. Effort remained constant across sessions at conceptual traps characterized by single-occasion transects (2 km), but varied across sessions for traps associated with grid cells incorporating multi-occasion transects. Mean effort across sessions and species for multi-occasion sites was 4.9 km (SD = 4.0, range = <1–21 km). The change in effective sampling area, log-likelihood, and *D* stabilized at a buffer width of 7.5 km, resulting in a state space of 3,663 $km^2$ (Figure 18).

Among the 12 capture models for *g0* and σ, the top kit fox model included variation among sessions and a trend in capture parameters within sessions (Table 15). The next closest model was >23 ΔAICc from the top model, indicating relatively little or no support (Table 15). Results aligned with capture probabilities estimated with non-spatial models (Table 14). We attempted to fit 24 models for density. Models containing the covariate distance to nearest water failed to converge; we rescaled this parameter to mean = 0 and standard deviation = 1, but convergence still failed. We successfully fit 14 models (Appendix 5.5 in Lonsinger 2015). The null model (i.e., D ~ session) received the greatest support, with the next closest model having a ΔAICc >136 (Appendix 5.5 in Lonsinger 2015). Due to the overwhelming support for the top model, we used *D* from this model. Kit fox *D* was similar across sessions (0.018–0.022 animals/$km^2$; Table 16). Derived estimates of kit fox abundance from SECR models were slightly higher than estimates from robust design non-spatial models across three sessions, but confidence intervals suggested that SECR and non-spatial estimates were similar (Figure 20).

**Table 14. Model-averaged estimates of capture probability (*p*) and unconditional standard error (SE) produced by program MARK by sex for kit foxes (*Vulpes macrotis*) surveyed with noninvasive genetic fecal sampling over two winter (W) and two summer (S) sessions in western Utah, USA, 2013–2014. Behavioral response was not expected with noninvasive sampling and thus recapture probability (*c*) was modeled as *p* = *c*.**

| | | Male | | Female | |
|---|---|---|---|---|---|
| Session[a] | Occasion[b] | *p* | SE | *p* | SE |
| Winter 2013 | 1 | 0.207 | 0.068 | 0.207 | 0.069 |
| | 2 | 0.236 | 0.064 | 0.236 | 0.065 |
| | 3 | 0.414 | 0.074 | 0.414 | 0.075 |
| | 4 | 0.536 | 0.093 | 0.536 | 0.094 |
| Summer 2013 | 1 | 0.432 | 0.094 | 0.431 | 0.095 |
| | 2 | 0.369 | 0.072 | 0.368 | 0.074 |
| | 3 | 0.322 | 0.081 | 0.321 | 0.083 |
| Winter 2014 | 1 | 0.489 | 0.074 | 0.489 | 0.074 |
| | 2 | 0.413 | 0.057 | 0.413 | 0.057 |
| | 3 | 0.373 | 0.084 | 0.373 | 0.084 |
| | 4 | 0.259 | 0.048 | 0.259 | 0.048 |
| | 5 | 0.186 | 0.053 | 0.186 | 0.053 |
| Summer 2014 | 1 | 0.276 | 0.088 | 0.272 | 0.087 |
| | 2 | 0.368 | 0.099 | 0.363 | 0.097 |
| | 3 | 0.415 | 0.088 | 0.409 | 0.087 |
| | 4 | 0.408 | 0.096 | 0.403 | 0.095 |

[a]Sessions represent primary sampling periods within a robust design.

[b]Occasions represent secondary sampling periods within a robust design.

**Table 15. Ranking of multi-session spatially-explicit capture models with parameters *g0* and σ (which jointly describe capture probability) fit for kit foxes (*Vulpes macrotis*) in western Utah, USA, based on Akaike's Information Criterion with small sample size correction (AICc). Each model is ranked based on ΔAICc (Δi = AICci – AICcmin), where K = number of parameters, $w_i$ = Akaike weight, and LL = log-likelihood. Across models, T = trend, t = time-varying, and session = primary sampling periods. Only the top four models and the null model are presented.**

| | | Kit fox | | | | |
|---|---|---|---|---|---|---|
| Model[a,b] | | K | $AIC_c$ | $\Delta AIC_c$ | $w_i$ | LL |
| *g0* ~ T*session | $\sigma$ ~ T*session | 20 | 3146.569 | 0 | 1 | -1550.348 |
| *g0* ~ t*session | $\sigma$ ~ t*session | 44 | 3169.765 | 23.196 | 0 | -1524.244 |
| *g0* ~ t+session | $\sigma$ ~ t+session | 20 | 3178.621 | 32.052 | 0 | -1566.373 |
| *g0* ~ T+session | $\sigma$ ~ T+session | 14 | 3215.218 | 68.649 | 0 | -1592.200 |
| *g0* ~ session | $\sigma$ ~ session | 12 | 3220.637 | 74.068 | 0 | -1597.285 |

**Table 16. Estimates of density (*D*) and standard error (SE) for kit foxes (*Vulpes macrotis*) over two winter (W) and two summer (S) sessions in western Utah, USA, 2013–2014. Estimates are based on spatially explicit capture-recapture models implemented with the R package 'secr'.**

| | Kit fox[a,b] | |
|---|---|---|
| Session | $\widehat{D}$ | SE |
| W 2013 | 0.018 | 0.003 |
| S 2013 | 0.019 | 0.003 |
| W 2014 | 0.022 | 0.003 |
| S 2014 | 0.020 | 0.003 |

[a]Density ~ session, *g0* ~ T*session, $\sigma$ ~ T*session (T = trend).

[b]Based on half-normal detection function where *g0* and $\sigma$ jointly describe capture probability.

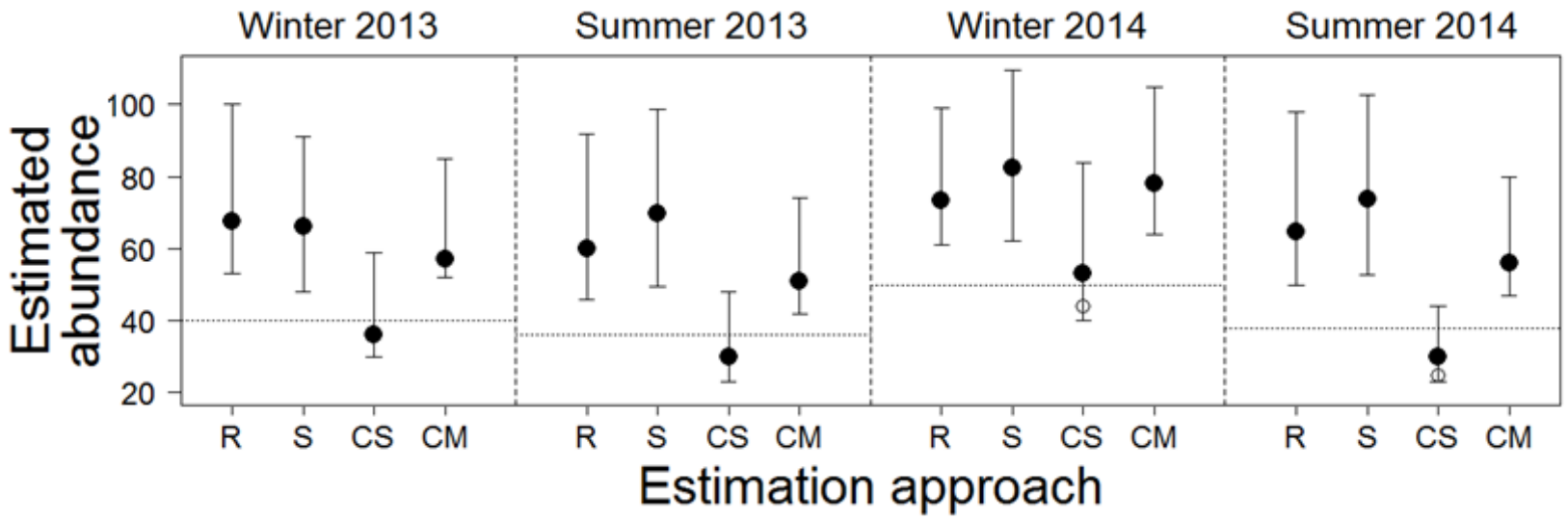



**Figure 20. Estimated abundances and 95% confidence intervals for kit foxes (*Vulpes macrotis*) in western Utah over four sessions, 2013–2014. Multiple estimators were used including robust design non-spatial Huggins closed-capture models (R), multi-session spatially explicit capture-recapture models (S), and two formulations of two-innate rates capture with replacement models based on single-occasion (CS) and multi-occasion (CM) sampling. Open circles represent capture with replacement point estimates under an equal capture model, where likelihood ratio tests failed to reject equal capture. The dashed horizontal line indicates the number of unique individuals identified within each session based on nuclear DNA.**

*Capture with replacement analysis*

We identified 103 transects of equal effort for CAPWIRE analyses. For the single-occasion formulation, total sampling effort was equal across sessions (Table 17). Effort varied among sessions for the multi-occasion formulation, reflecting variation in occasions (Table 17). Across sessions, we identified only 21–30 kit foxes, when considering only transects contributing to single-occasion CAPWIRE estimates (Table 17). With single-occasion CAPWIRE surveys, we detected 55.3–62.5% of the minimum number known alive (MNKA) for kit foxes, within each session. When considering multi-occasion CAPWIRE surveys, we identified ≥86% of known kit foxes within each session (Table 17). We failed to detect a greater proportion of the MNKA due to the reduction in occasions (27.8–36.8%), than due to the decreased transect length associated with identifying nested transects (7.5–13.9%). The number of captures per kit fox ranged from 1.6–2.3 for single-occasion and 1.9–2.4 for multi-occasion sampling.

Likelihood-ratio tests rejected the ECM for multi-occasion models across sessions (all $P < 0.02$); for single-occasion models, the ECM was rejected for 2013 sessions (both $P < 0.03$), but was not rejected for 2014 sessions (both $P > 0.1$). Because likelihood ratio tests may fail to reject the ECM when sample sizes are small and capture heterogeneity is present (Miller et al. 2005), we report results under the TIRM, but include ECM point estimates in Figure 20 where they were supported.

Kit fox abundance estimates from CAPWIRE were generally lower than those from robust design non-spatial models and SECR models (Figure 20). From single-occasion CAPWIRE models, kit fox estimates were substantially lower (27.5–59.2%) than multi-session estimates, ranging from 30–53 (Figure 20); estimated abundance was lower than the MNKA in three sessions. Generally, kit fox single-occasion CAPWIRE estimates had higher precision than alternative estimation approaches, and 95% confidence intervals failed to overlap multi-session point estimates in all but one session (Figure 20). Kit fox multi-occasion CAPWIRE estimates were lower (5.3–27.0%) than multi-session estimates (with one exception, winter 2014), but confidence intervals overlapped considerably (Figure 20).

*Comparing multi-session estimators of abundance*

Combining NGS and capture-recapture methods can yield reliable population estimates (e.g., Puechmaille and Petit 2007, Stenglein et al. 2010b). Advances in SECR models have expanded the modeling framework available to practitioners and allow for the estimation of both density and the effective sampling area under a unified framework (Borchers and Efford 2008, Royle et al. 2014). True abundance is unknown for our target populations and we cannot explicitly infer bias for each estimator. Abundance estimates from robust design non-spatial and multi-session SECR models showed high levels of agreement for both species and across sessions (Figure 20). In general, SECR estimates were slightly higher than robust design non-spatial estimates. Blanc et al. (2013) found SECR models tended to overestimate abundance for small populations (defined as $N = 10$), but produced estimates closer to the true abundance for larger populations (defined as $N = 50$). Our MNKA and abundance estimates suggested that kit fox populations were >50 individuals. Individual heterogeneity in capture, if unaccounted for, can bias abundance estimates downward (Otis et al. 1978, White et al. 1982). Spatial models address variation resulting from an individual's proximity to survey sites, a form of heterogeneity not accounted for by non-spatial models (Borchers and Efford 2008, Royle et al. 2014). Thus, lower abundance estimates from non-spatial models may be the result of this capture heterogeneity.

**Table 17. Total survey effort, number of unique kit foxes (*Vulpes macrotis*) captured, and proportion male (M) that were considered when employing capture with replacement models to estimate population abundances over two winter (W) and two summer (S) sessions in western Utah, USA, based on single-occasion (Single) and multi-occasion (Multi) sampling schemes.**

| | Total Effort (km)[a] | | Number of individual detected (M) | |
|---|---|---|---|---|
| Session | Single-occasion | Multi-occasion | Single-occasion | Multi-occasion |
| W 2013 | 206 | 464 | 25 (0.68) | 37 (0.68) |
| S 2013 | 206 | 378 | 21 (0.57) | 31 (0.52) |
| W 2014 | 206 | 550 | 30 (0.53) | 45 (0.64) |
| S 2014 | 206 | 464 | 21 (0.52) | 35 (0.49) |

While both Huggins closed-capture models and SECR models assume population closure (Otis et al. 1978, Royle et al. 2014), SECR models relax the assumption by taking into account an animal's activity center. Population losses or gains that violate closure assumptions can negatively or positively bias estimates, respectively (Kendall 1999). Closure tests suggested kit fox population losses in later sampling sessions, and this could have resulted from increased reproductive behavior (i.e., denning) in winter and initiation of juvenile dispersal in summer. Closure test results should be viewed with caution though, as they assume no individual heterogeneity in *p* and closure is rejected at high rates in closed populations when heterogeneity exists (Stanley and Burnham 1999). We observed a similar magnitude in the differences between abundance estimates from robust design non-spatial models and SECR models in 2013 and 2014 for kit foxes. This, combined with knowledge that concurrent research involving telemetered kit foxes did not detect any movements to beyond our study extent (B. Kluever, personal communication), lead us to believe the kit fox population was effectively closed.

Non-spatial models do not account for 'holes' in the sampling frame (Williams et al. 2002, Efford and Fewster 2013), and this may also contribute to the lower abundance estimates resulting from non-spatial models. Our random sampling at the landscape scale resulted in several holes within our sampling frame (Figure 18), from which animal's likely had low (or possibly zero) *p* due to their proximity to transects. By accounting for proximity to animal activity centers, SECR models effectively handle holes (Borchers and Efford 2008, Royle et al. 2014). The detection function we employed assumed a circular home range (Efford et al. 2009); kit fox typically have circular home ranges (Koopman et al. 2000).

We observed similar levels of precision between non-spatial and spatial models. Spatial models are able to utilize more of the capture data (i.e., do not require collapsing of spatially disparate captures to a binary response) and often have higher precision than non-spatial models (Sollmann et al. 2011, Blanc et al. 2013). Trap spacing and sampling intervals may influence precision of SECR models though, as greater inter-trap spacing and shorter sampling intervals

likely reduce opportunities for spatially disparate recaptures within an occasion. As the probability of spatially disparate recaptures decreases, capture histories for spatial and non-spatial models converge. Our average inter-trap distance (2.7 km) may have limited the opportunity for spatial recaptures within an occasion.

*Comparing multi-session and capture with replacement estimators*

Multi-session models (i.e., robust design non-spatial models and SECR models) produced relatively consistent results, and we used these as a standard to evaluate the performance of single-occasion and multi-occasion CAPWIRE estimators. The MNKA nearly always underestimates abundance (Mills et al. 2000), and therefore we regard estimates at or below the MNKA as biased. In practice, limited resources often force managers to seek out cost-efficient sampling strategies. Consequently, there has been considerable interest in single-occasion sampling schemes, which have practical advantages (e.g., ease of implementation, lower cost; Miller et al. 2005, Petit and Valiere 2006, Williams et al. 2009). Reliable estimates have been reported for a range of taxa using CAPWIRE (Petit and Valiere 2006, Puechmaille and Petit 2007, Robinson et al. 2009, Stenglein et al. 2010b), but in some cases, CAPWIRE estimates do not align with alternative estimates (Ruell et al. 2009, Williams et al. 2009, Stansbury et al. 2014). Simulations have suggested that single-occasion sampling can produce abundance estimates as reliable as multi-occasion sampling when the number captures per individual is >1.7 (Miller et al. 2005, Petit and Valiere 2006, Stenglein et al. 2010b). Our captures per individual exceeded was ≥1.6 across sessions. Still, single-occasion CAPWIRE estimates were substantially lower than multi-session estimates for both species across sessions. Single-occasion kit fox estimates fell below the MNKA for three of four sessions; all estimates fell below the MNKA when employing the ECM where it was supported (Figure 20).

The CAPWIRE model assumes independence among captures and equal sampling effort (Miller et al. 2005). Independence among captures may be violated when individuals are captured multiple times within a site and restricting recaptures to spatially disparate sites can reduce this concern (Stenglein et al. 2010b). Placing restrictions on how recaptures are defined, however, can reduce already limited datasets available for rare carnivores and will likely result in fewer captures per individual (Stansbury et al. 2014). Consequently, many researchers opt to include all captures (Miller et al. 2005, Williams et al. 2009, Stansbury et al. 2014) as we have, and this may bias results and artificially inflate precision. CAPWIRE models are based on a simple urn model (Miller et al. 2005) and may best apply to sampling situations that mimic this, such as sampling where animals congregate (e.g., rendezvous sites, breeding grounds, roosting colonies). Our sampling was relatively dispersed and we did not target animal concentration areas. Temporal variation in space-use may limit the number of individuals available for capture during a single occasion, biasing CAPWIRE estimates (Kendall 1999). Ensuring that >1 single-occasion transect is within each potential home range may alleviate this concern, but may be impractical or restrict the spatial extent that can be surveyed. Alternatively, combining the results from multiple occasions, while accounting for variable effort to meet model assumptions, may increase the probability of capturing individuals with temporal variation in space-use. Our data suggests that this may be the case with kit foxes, as we substantially increased the number of individuals captured ≥1x by increasing the number of occasions and resulting estimates were generally more similar to those from multi-session estimators.

Across estimators and species, we expected to observe an increase (or pulse) in summer abundance, relative to winter abundance, resulting from annual reproduction. We failed to detect these reproductive pulses and this likely reflects the precision of estimates. Alternatively, capture probability of juveniles may be lower along linear features. If nightly foraging events by juveniles are shorter in distance than adults, juveniles may have lower probability of encountering survey transects. If foraging events are shorter in duration or less frequent, juveniles may be less likely to deposit scats along a transect, even if one is encountered. One limitation of scat sampling is the inability to determine the age of individuals, and we therefore were unable to assess the potential for such differences.

*NGS-OM sampling and species identification*

Sampling effort was constant across sessions, with 103 sites each being surveyed via four 500 m transects per session, resulting in 824 km of surveys (206 km/session). From the samples collected, 1,702 samples contributed to the occupancy analysis (Table 18). Across sessions, naïve estimates of coyote occupancy were >0.7 in all but the first session and kit fox occupancy was ≤0.3 (Table 18, Figure 21, Figure 22).

**Table 18. Number of carnivore scats identified as kit fox (*Vulpes macrotis*), coyote (*Canis latrans*), or a nontarget carnivore (NTC; included domestic dog, red fox [*V. vulpes*], bobcat [*Lynx rufus*], and cougar [*Puma concolor*]) based on mitochondrial DNA (mtDNA) species identification, mtDNA amplification success rates, and naïve occupancy (ψ) for kit foxes and coyotes. Scat surveys were conducted within 103 sites (each 6.25 km2) over four sessions (winter 2013, su`mmer 2013, winter 2014, and summer 2014) in Utah, USA. Mixed samples contained DNA from >1 species.**

| | Number of carnivore scats | | | | | | mtDNA success rate | Naïve ψ | |
|---|---|---|---|---|---|---|---|---|---|
| Session | Total | Coyote | Kit fox | NTC | Mixed | Failed | | Coyote | Kit fox |
| 1 | 218 | 136 | 60 | 3 | 2 | 17 | 92.2% | 0.52 | 0.21 |
| 2 | 628 | 340 | 97 | 27 | 5 | 159 | 74.7% | 0.72 | 0.28 |
| 3 | 363 | 247 | 87 | 7 | 5 | 17 | 95.3% | 0.74 | 0.30 |
| 4 | 493 | 362 | 65 | 11 | 6 | 49 | 90.1% | 0.73 | 0.23 |
| Total | 1,702 | 1,085 | 309 | 48 | 18 | 242 | 85.8% | | |

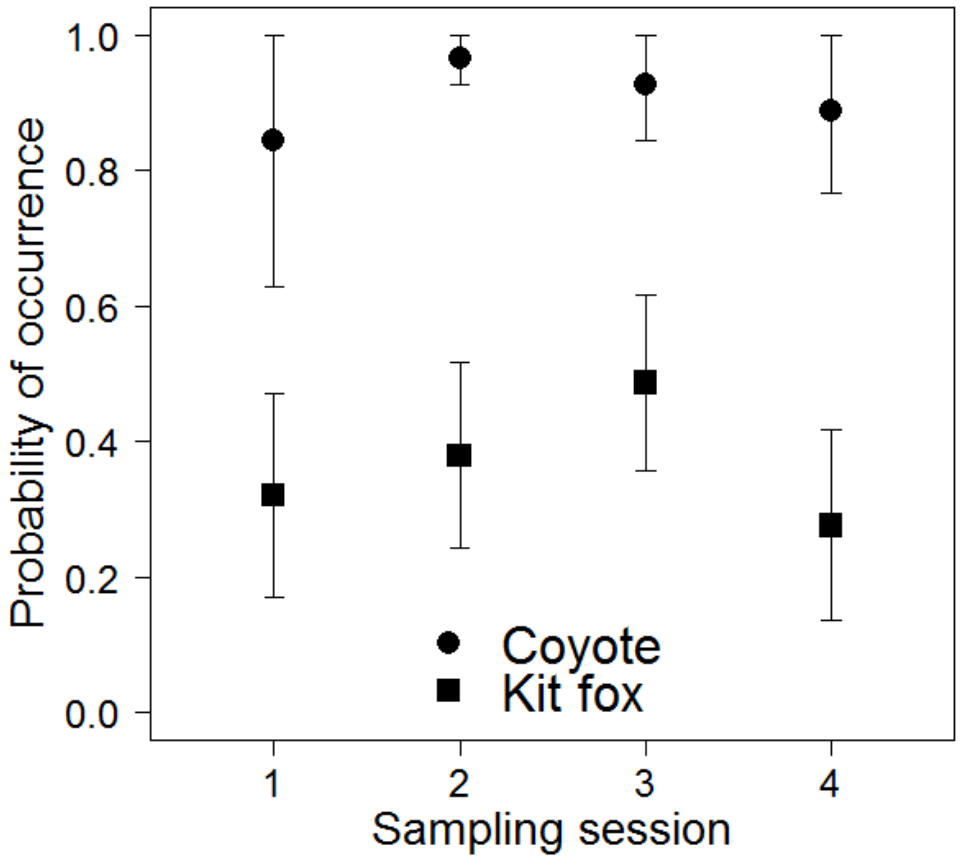



**Figure 21. Initial (session 1) and derived (sessions 2–4) probabilities of occurrence with 95% confidence intervals for coyotes (*Canis latrans*) and kit foxes (*Vulpes macrotis*) over four sessions in Utah, USA, 2013–2014. Probability of occurrence is plotted based on the best-fit models for each species and the median proportion of shrubland and woodland cover (13.0%).**

*NGS-OM site and survey characteristics*

Soil for the majority of sites was predominantly silt (46) or fine sand (36), with fewer sites being primarily blocky loam (12) or gravel (9). Mean %SW for sites was 21.8% (± 2.25 SE), though the distribution was right skewed (median = 13.0%, range = 0–97%). Distance to nearest water ranged from 0.2–12.4 km (mean = 3.96 km ± 0.28 SE). The mean number of water sources within 2.5 and 5 km was 0.54 (± 0.08 SE) and 1.95 (± 0.02 SE), respectively; 64 sites had no water within 2.5 km (median = 0, range = 0–5) and 28 had no water within 5 km (median = 2, range = 0–7; Figure 22). Mean road density across sites was 1.17 km/km2 (± 0.05 SE). Over half (55%) of 500 m transects were along unmaintained two-track roads, and 31% and 14% were along single-lane and two-lane gravel roads, respectively. Snow was present during surveys at 92% (95) and 49% (50) of the sites in winter 2013 and 2014, respectively.

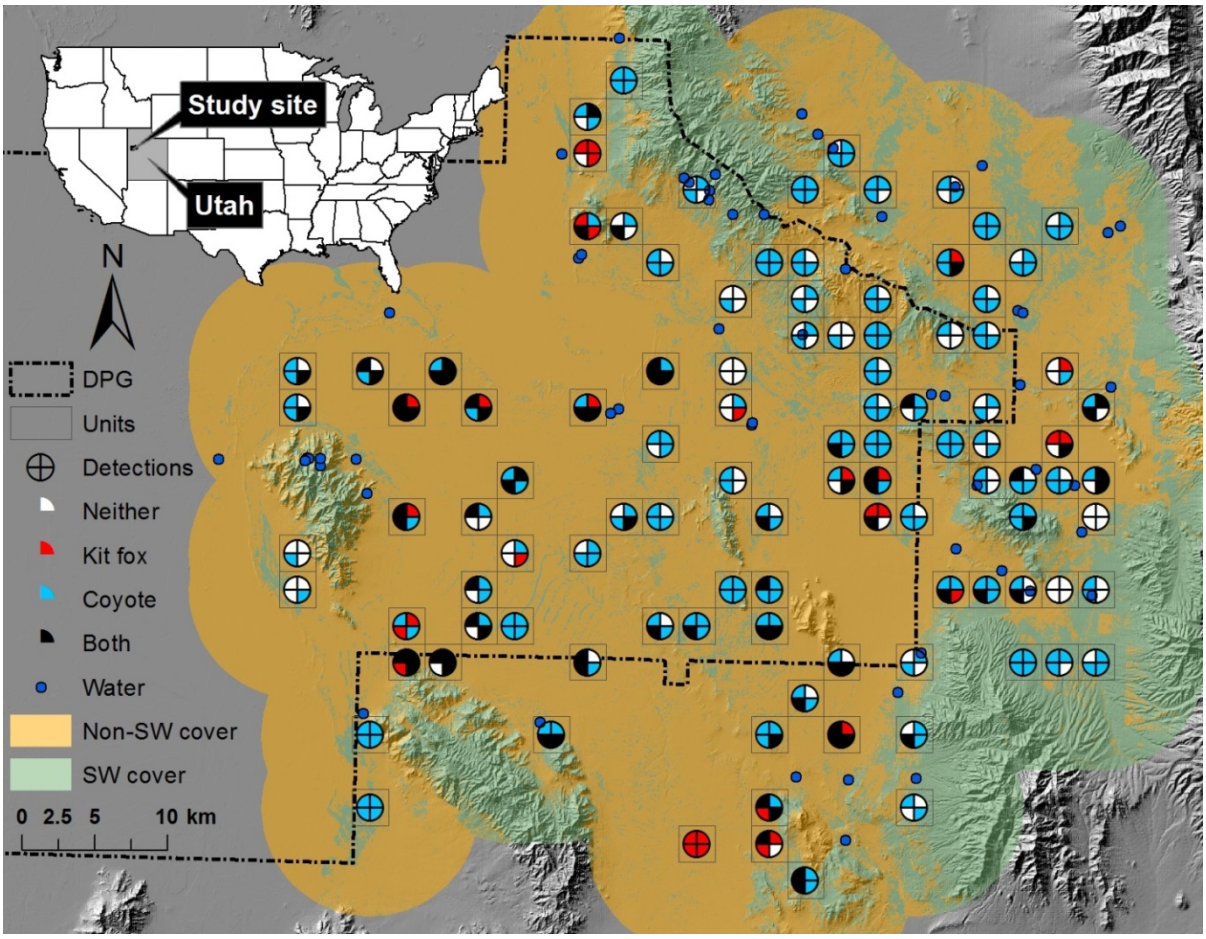



**Figure 22. Location of 103 sites (units; each 6.25 km$^2$) surveyed for coyotes (*Canis latrans*) and kit foxes (*Vulpes macrotis*) over four sessions in Utah, USA, 2013–2014. The pie charts indicate whether kit fox, coyote, both, or neither was detected during winter 2013 (upper right), summer 2013 (lower right), winter 2014 (lower left), and summer 2014 (upper left). Habitat classifications display the distribution of shrubland and woodland cover (SW) versus areas with lower (e.g., grasslands) or more sparse (e.g., playa) vegetative cover, within 7.5 km of sites.**

*NGS-OM dynamic models*

Coupling noninvasive sampling with co-occurrence occupancy modeling offers an efficient framework to investigate the interactions of intraguild species (e.g., kit foxes and coyotes, Robinson et al. 2014). To our knowledge, our study is the first to employ NGS and dynamic occupancy models to explore this type of system. As discussed in Section 5.5.1, we intended to use a multi-species (i.e., co-occurrence) framework to investigate the influence of coyotes on patterns of kit fox occupancy. Dynamic models for coyote occupancy and the resulting estimates are described in detail in Lonsinger (2015). In summary, best-fit models produced estimates of coyote $\psi$ that were not significantly different than 1 (Figure 21). Because coyotes were widespread, there was insufficient heterogeneity in coyote occupancy to effectively use a co-occurrence framework to evaluate species interactions. As previously described (Section 5.5.1),

we instead incorporated coyote activity as a covariate in kit fox dynamic occupancy models and demonstrated how variation in coyote sign among and within sites can be exploited to investigate the influence of intraguild pressures on the spatial dynamics of kit foxes using a single-species dynamic modeling framework.

The best-fit global kit fox model included water characterized as the distance to nearest water and an ordinal relationship between road types (Appendix 6.3 in Lonsinger 2015); these formulations for each covariate carried the highest cumulative weights (Table 19). The influence of coyotes on kit foxes was best characterized as the total number of coyote scats detected (coyote activity) at both the site and transect levels (Table 19, Appendix 6.3 in Lonsinger 2015). The best-fit detection model suggested that kit fox *p* was positively related to transect-level coyote activity ($\beta = 0.20 \pm 0.06$ SE). Road type ($\beta = 0.23 \pm 0.15$ SE) was present in the best-fit kit fox *p* model, but had a negligible relationship with the $\beta$ estimate's 95% CI overlapping zero. Mean kit fox *p* was similar across sessions (winter: 2013 = 0.24 ± 0.02 SE, 2014 = 0.25 ± 0.02 SE; summer: 2013 = 0.26 ± 0.02 SE, 2014 = 0.26 ± 0.02 SE).

Initial kit fox $\psi$ was substantially lower than coyote $\psi$ (Figure 21). The best-fit model indicated %SW had a strong negative ($\beta = -13.46 \pm 3.98$ SE) influence on kit fox $\psi$; this relationship was the inverse to the relationship between coyotes and %SW (Figure 23; Lonsinger 2015). The cumulative weight for %SW was high and no other predictors carried substantial weight (Table 19; Appendix 6.3 in Lonsinger 2015). Among models for kit fox dynamic parameters (Appendix 6.3 in Lonsinger 2015), the best-fit model suggested that site-level coyote activity positively influenced both $\varepsilon$ ($\beta = 0.97 \pm 0.45$ SE) and $\gamma$ ($\beta = 0.23 \pm 0.15$ SE), though the effect on $\gamma$ was weak, with 95% CIs for $\beta$ overlapping zero. Additionally, $\varepsilon$ varied temporally and soil type influenced $\gamma$ (Appendix 6.3 in Lonsinger 2015). Coyote activity carried substantial model weight for $\varepsilon$ (Table 19). For $\gamma$, soil had the greatest influence based on cumulative model weights, followed by coyote activity (Table 19). Derived estimates of kit fox $\psi$ from the best-fit model were similar across sessions (Figure 21). As predicted based on the intraguild relationship between coyotes (i.e., the intraguild predator) and kit foxes (i.e., the intraguild prey), kit fox $\psi$ was more stable in sites with lower coyote activity (Figure 24).

*Patterns of kit fox occurrence*

Canids commonly employ spatial partitioning to facilitate coexistence, with dominant predators conforming to predictions of the resource availability hypothesis (Ernest et al. 2000, Blaum et al. 2007), while suborindate predators occupy habitats that minimize risk of intraguild predation, aligning with expectations of intraguild predation theory and mesopredator suppression (Soulé et al. 1988, Heithaus 2001).

Shrubland and woodland habitats at our study region tended to support greater mammalian prey abundance and diversity than alternative habitats (Arjo et al.2007, Kozloswki et al. 2012), and the vegetative structure may have provided greater thermal cover for large-bodied predators (Blaum et al. 2007). Coyote occupancy declined precipitously when shrubland and woodland cover dropped below 20% (Lonsinger 2015), while kit fox occupancy displayed an inverse relationship (Figure 23), suggesting broad scale habitat partitioning. These results aligned with previous work on coyotes and foxes at DPG (Kozlowski et al. 2012) and elsewhere (Nelson et al. 2007, Thompson and Gese 2007, Robinson et al. 2014). We were unable to detect an influence of site-level coyote activity on static kit fox occupancy.

**Table 19. Cumulative Akaike model weights ($\Sigma w_i$) for predictors of kit fox (*Vulpes macrotis*) detection, occupancy, and probability of local extinction and colonization across 103 sites in western Utah, USA, 2013–2014, from the complete model set used to evaluate each parameter (see Lonsinger 2015 for full model sets by parameter).**

| Global | | Detection | | Occupancy | | Extinction | | Colonization | |
|---|---|---|---|---|---|---|---|---|---|
| Pred | $\Sigma w_i$ | Pred | $\Sigma w_i$ | Pred | $\Sigma w_i$ | Pred | $\Sigma w_i$ | Pred | $\Sigma w_i$ |
| DistW | **0.70** | CA | **0.97** | SW | **0.99** | CS | **0.95** | Soil | **0.80** |
| W5 | 0.18 | RTO | **0.77** | CS | 0.37 | t | **0.94** | CS | **0.56** |
| W2 | 0.11 | Date | 0.32 | RD | 0.25 | SW | 0.32 | DistW | 0.40 |
| RTO | **0.83** | Snow | 0.28 | DistW | 0.24 | DistW | 0.28 | SW | 0.40 |
| RTC | 0.17 | RD | 0.26 | Soil | 0.16 | RD | 0.26 | RD | 0.30 |
| CS | **0.77** | Sun | 0.25 | | | Soil | 0.16 | t | 0.26 |
| CT | 0.23 | t | 0.18 | | | | | | |
| CA | **0.78** | | | | | | | | |
| CP | 0.22 | | | | | | | | |

Bold indicates predictors present in the best-fit model. Global represents the evaluation of different formulations for water, road type, and site and transect level coyote activity to identify a single global model for each species. Predictors: DistW = distance to nearest water source (km), W2 = number of water sources within 2.5 km of site center, W5 = number of water sources within 5 km of site center, RTO = ordinal road type coding, RTC = categorical road type coding, RD = road density (km/km2), Snow = presence or absence, Sun = difference between survey time and solar noon, Date = days since surveys were initiated within sampling session, SW = proportion of land cover attributable to shrubland and woodland habitats, Soil = categorical classification of the majority soil type for a site (four types: silt, fine sand, blocky loam, or gravelly), CS = total number of coyote scats detected at the site level, CT = total number of transects on which coyotes were detected at the site level, CA = number of coyote scats detected at the transect level, CP = binary detection (1) or non-detection (0) of coyotes at the transect level, t = time-varying.

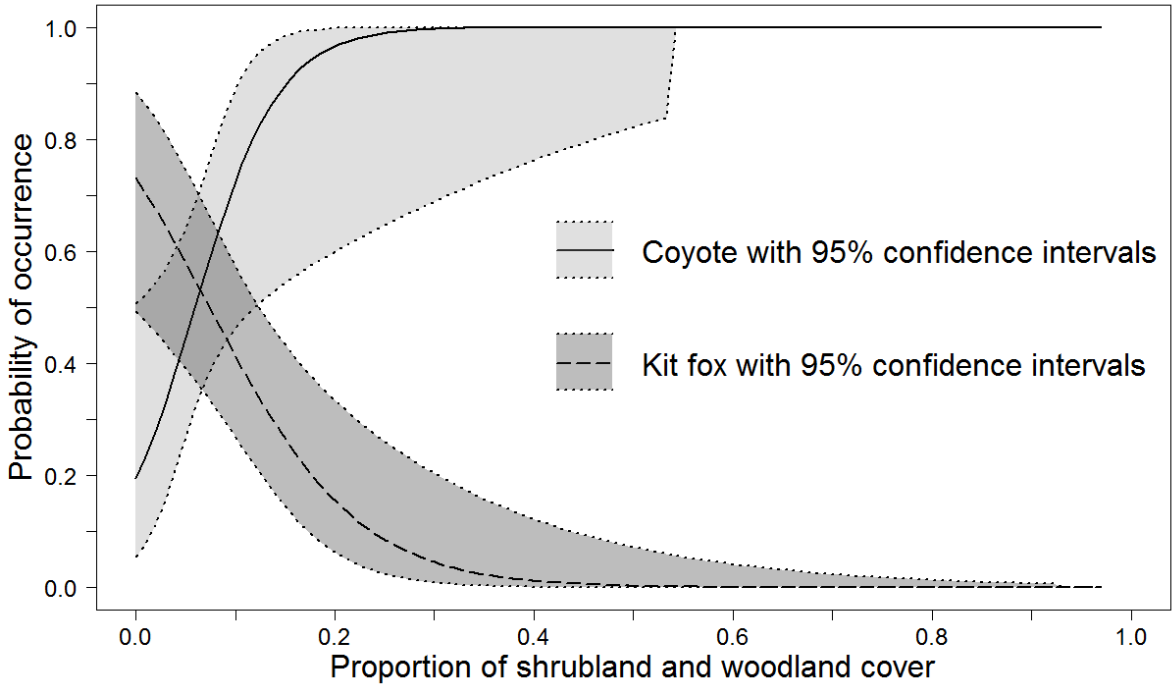



**Figure 23. Initial probability of occurrence with 95% confidence intervals for coyotes (*Canis latrans*) and kit foxes (*Vulpes macrotis*) as a function of shrubland and woodland cover in Utah, USA, 2013–2014. Probability of occurrence is plotted based on the best-fit model for each species and mean covariate values.**

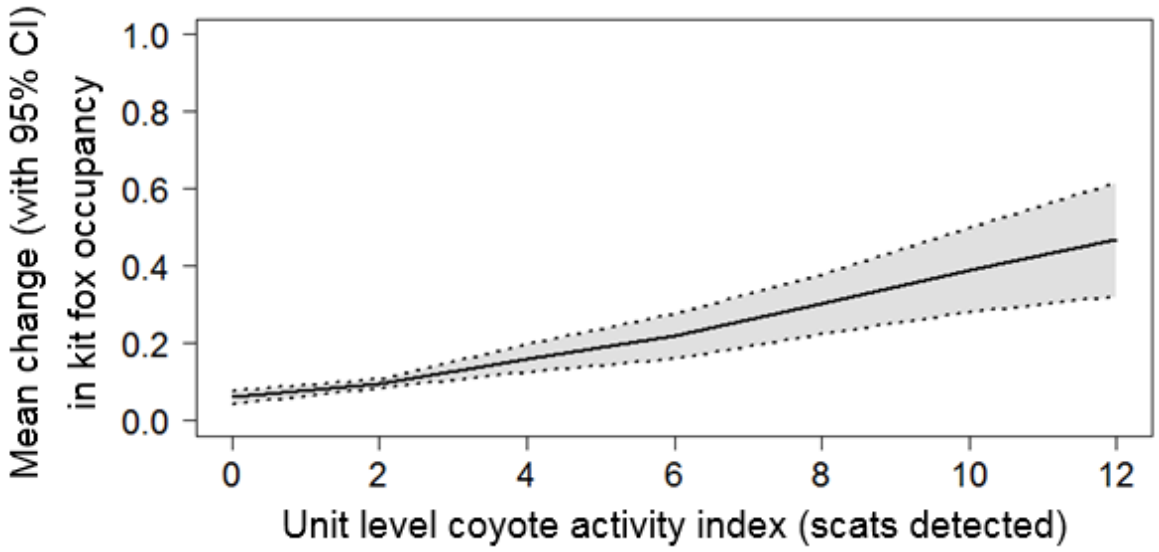



**Figure 24. Mean change in probability of occurrence with 95% confidence intervals (CI) across sessions for kit foxes (*Vulpes macrotis*) as a function of coyote activity, in Utah, USA, 2013–2014. Mean change in occupancy is based on the best-fit models and mean covariate values.**

Investigations of canid intraguild predation systems have focused primarily on static occupancy (e.g., Robinson et al. 2014), but elucidating drivers of local extinction and colonization can improve our understanding of how covariates and species interactions drive space use (MacKenzie et al. 2003). Observed occupancy states result from preceding patterns of local extinction and colonization. As predicted, kit fox probability of local extinction was elevated across sites with higher coyote activity. Intraguild predation theory predicts local extinctions of subordinate species may be regulated by a dominant predator, and that intraguild predation effects will be more acute when the two have high dietary overlap (Polis et al. 1989, Holt and Polis 1997). Dietary overlap of coyotes and kit foxes was high at DPG (Kozlowski et al. 2008) and when sympatric, coyote predation accounts for a significant proportion of kit fox mortalities (56–78%; Ralls and White 1995, Nelson et al. 2007, Kozlowski et al. 2008). Consequently, local extinction may result from a decreased ability to avoid intraguild predation pressures at sites with higher coyote activity.

Kit foxes utilize burrows year-round to provide relief from environmental conditions and predators (Arjo et al. 2003). Thus, it was not surprising that silty soil, which facilitates burrow excavation (Egoscue 1956), promoted kit fox colonization. At DPG, Egoscue (1962) indicated kit foxes utilized primarily silt and/or clay soils, and Dempsey et al. (2015) found a negative relationship between kit fox presence and blocky loam soil.

*Patterns of fine-scale kit fox space use*

Coexistence among intraguild predators often requires subordinate species to adjust their activity patterns or fine-scale space use; few empirical examples of such behavioral responses exist for mammalian systems. Vanak et al. (2013) found that predators were aware of competitors at various spatial scales and subordinate species adjusted movement patterns in the presence of dominant predators. Similarly, cape fox (*V. chama*) habitat selection did not differ in the presence of jackals at broad scales, but they had atypically large home ranges in the presence of jackals, presumably to facilitate jackal avoidance during foraging (Kamler et al. 2013).

Recent research at our study site found that among survey methods, scat surveys produced results that most closely aligned with minimum known canid abundance (Dempsey et al. 2014). Thus, we assumed that the number of coyote scats detected along each transect (i.e., spatial replicate) was reflective of coyote activity. The diets and nightly activity patterns of kit foxes and coyotes overlap significantly at DPG, and it has been suggested that broad scale habitat partitioning and safety matching facilitates coexistence (Arjo et al. 2007, Kozlowski et al. 2008, 2012). Hall et al. (2013) supported the lack of temporal separation between species, but failed to detect spatial partitioning. The scale of inference is essential to understanding patterns of co-occurrence and space use. At broad scales, our results align with those of Kozlowski et al. (2012): coyote occupancy increased with increasing shrubland and woodland cover, presumably reflecting resource matching, while kit foxes occupancy was inversely related to shrubland and woodland cover, reflecting patterns consistent with safety matching. At finer scales, our results are consistent with those of Hall et al. (2013) indicating a lack of spatial separation: kit foxes space use was highest where coyote activity was highest, suggesting that within their home ranges, kit foxes may use riskier habitats to secure sufficient resources (i.e., resource matching). Similar patterns were recently observed in New Mexico, where kit foxes exhibited broad scale spatial partitioning with coyotes, but were more likely to occupy sites with, than without, coyotes (Robinson et al. 2014). The nature of our sampling design (i.e., noninvasive with no temporal

replication within sessions) precludes any inference on fine-scale temporal partitioning. Though nightly activity periods were similar for kit foxes and coyotes (Kozlowski et al. 2008), temporal partitioning may be occurring at an intermediate temporal scale, with kit fox avoiding coyotes by using similar areas but doing so over different periods (e.g., over different nights).

### 5.6.2 Barry M. Goldwater Range and Cabeza Prieta NWR, Arizona

From 13 drinkers, we collected 730 samples in 2013 and 980 samples in 2014 and extracted 634 and 692 samples in 2013 and 2014. We also collected and extracted 79 non-drinker samples in 2014 (Table 20). Four single-session drinkers had no indication of recent pronghorn use. In 2013 and 2014, 75% (n = 474) and 72% (n = 555) of pronghorn samples achieved a consensus genotype for individual ID. Samples classified as freshest (F1) had the highest individual ID success rates. Individual ID success rates increased in later sessions in 2013, but were generally consistent across sessions in 2014 (Tables 21, 22, 23). We predicted slightly lower success rates in session 1 as we did not clear fecal pellets from sites prior to our first sampling session, and thus some pellets may have been more than 1 week old. In 2013 but not 2014, success rates increased in later sessions. We don't believe this affects the population closure assumption as our pilot studies indicated low nDNA PCR success rates (2–28%) by day 14 and 0% by day 60 (Woodruff et al. 2015, S.P. Woodruff, unpublished data).

**Table 20. Age group, freshness classification, and number of Sonoran pronghorn fecal samples collected at single and multi-session drinkers.**

| | Multi-session sites (Single-session sites) | | | | |
|---|---|---|---|---|---|
| | Adults | Fawns | F1 | F2 | F3 |
| 2013 | 635 (35) | 58 (2) | 55 (2) | 343 (7) | 295 (28) |
| 2014 (drinker) | 787 (36) | 211 (0) | 54 (4) | 335 (9) | 609 (23) |
| 2014 (non drinker) | 24 | 1 | 4 | 2 | 19 |

F1 = freshest; F2 = less fresh; F3 = oldest

The average number of alleles per locus was 5.8 (range = 4–9; SD = 1.75) and mean expected heterozygosity of the 10 loci was 0.65 (range = 0.60–0.78; SD = 0.10). Allelic dropout rates were higher than false alleles and overall genotyping error rates were slightly higher in 2014 (Table 21). Our final dataset contained no individuals mismatched at only one locus and one pair of samples that mismatched at two out of 10 loci. We amplified these samples at an additional six loci resulting in four mismatches at the 16 loci, and they were determined to be two individuals.

**Table 21. Sonoran pronghorn fecal pellet samples collected and analyzed in 2013 and 2014 and genotyping error rates are provided per year and by freshness category.**

| Year | Freshness | # Collected | # Analyzed | Success[a] (%) | ADO[b] (%) | FA[c] (%) |
|---|---|---|---|---|---|---|
| 2013 | Total | 730 | 634 | 75 | 9.5 | 2.2 |
| | F1[d] | 57 | 57 | 96 | 10.4 | 2.1 |
| | F2 | 350 | 332 | 82 | 9.3 | 2.2 |
| | F3 | 323 | 245 | 68 | 9.5 | 2.3 |
| 2014[e] | Total | 1059 | 771 | 72 | 15.7 | 4.6 |
| | F1 | 62 | 57 | 86 | 10.8 | 3.4 |
| | F2 | 346 | 356 | 77 | 15.9 | 4.2 |
| | F3 | 651 | 358 | 69 | 16.4 | 5.3 |

[a] Individual ID success rates
[b] ADO = allelic dropout
[c] FA = false allele
[d] Freshness category: F1 = most fresh, F2 = moderately fresh, F3 = least fresh.
[e] Includes samples from drinker and non-drinker locations

**Table 22: Breakdown of fecal DNA individual ID success by year, single or multi-session, and freshness and sampling session. F1, F2, and F3 are freshness categories with F1 being the freshest and sessions are labeled S1–S3.**

| | Percent Success (n = # of samples) | | | |
|---|---|---|---|---|
| | Multi | | Single | |
| | 2013 (n) | 2014 | 2013 | 2014 |
| F1 | 96 (55) | 86 (43) | 100 (2) | 100(9) |
| F2 | 82 (258) | 77 (337) | 50 (5) | 69 (16) |
| F3 | 68 (158) | 69 (275) | 73 (15) | 68 (59) |
| S1 | 80 (114) | 77 (212) | 61 (17) | 75 (77) |
| S2 | 92 (124) | 71 (124) | --- | --- |
| S3 | 91 (126) | 74 (148) | --- | --- |

**Table 23. Individual ID success chi-square and p-values for pellet freshness and sampling session for Sonoran pronghorn fecal pellets. Asterisks represent a statistically significant result at p-value of 0.05. There was not a session 4 in 2014 which is represented by NA.**

| | 2013 | | 2014 | |
|---|---|---|---|---|
| | $\chi^2$ | p | $\chi^2$ | P |
| F1 to F2 | 6.26 | 2.21e-06* | 1.86 | 0.17 |
| F2 to F2 | 12.67 | 3.30e-04* | 4.99 | 0.03* |
| F1 to F3 | 16.54 | 4.76e-05* | 5.75 | 0.02* |
| ALL | 15.61 | 0.001* | 2.26 | 0.32 |
| S1 to S2 | 7.34 | 0.007* | 1.88 | .02* |
| S1 to S3 | 6.66 | 0.01* | 0.58 | 0.45 |
| S1 to S4 | 5.53 | 0.02* | NA | NA |
| S2 to S3 | 0.00 | 1.00 | .023 | 0.63 |
| S2 to S4 | | 0.50[a] | NA | NA |
| S3 to S4 | | 0.57[a] | NA | NA |

[a]Odds ratio from Fisher's Exact Test

**Table 24. Number of detections and number of unique individual Sonoran pronghorn identified in 2013 and 2014 with pronghorn sampled at nondrinker locations in 2014 shown in parentheses.**

| Year | | | | | |
|---|---|---|---|---|---|
| 2013 | | | Adult Male | Adult Female | Fawn |
| | #Detections | 474 | | | |
| | # Individuals | 91 | 50 | 24 | 17 |
| 2014 | #Detections | 555 | | | |
| | # Individuals | 127 | 69 (8) | 38 (1) | 20 (3) |

We had 474 detections of 91 individuals (50 adult males, 24 adult females, 17 fawns) in 2013 and 555 detections of 127 individuals (69 adult males, 38 adult females, 20 fawns) in 2014. Twenty-one of the individuals (8 adult males, 10 adult females, 3 fawns) in 2014 were detected at 9 non-drinker locations, 4 of which were also detected at drinker locations (Table 24). Sixty-three individuals detected in 2013 were also detected in 2014. The number of detections per individual (i.e., samples) in a year ranged from 1 to 32, and 33.7% and 26.0% were single detections in 2013 and 2014. At drinkers we detected 2.5–3 times more adult male than female samples, and the average number of detections per individual was 6.1 and 3.6 for adult males and females.

The top model was the same and included equal survival (φ) by sex and equal detection and redetection probabilities both varying by time and group (Table 25). Males had higher detection probabilities (range: 0.64–0.76) than females (range: 0.36–0.61) in all occasions across years (Figure 25).

At drinker locations, the models produced (summed) population estimates of 116 individuals (95% CI: 101–132) in 2013 and 121 individuals (95% CI: 112–132) in 2014. For all locations (2014 only), the population estimate was 144 individuals (95% CI: 132–157). Population estimates indicated a bias towards males with 1.4–1.6 times more adult males than adult females. When comparing annual population estimates from drinker locations, the results suggest little to no change in population size. As expected, the population estimate at the expanded geographic location was larger as more individuals were sampled.

*Comparing abundance estimators*

Our empirical data had increased precision with more sessions. The extra samples (n = 138; 2013 only) resulted in additional captures of 37 individuals including three individuals not previously identified. One “extra” individual was detected in session 2 only and two were caught in session 3 only. Population estimates changed only slightly with the inclusion of the extra samples (Table 26). The *capwire* estimates from session 3 only decreased 9.7%, while all other estimates stayed the same or marginally increased (2–6.5%) for both methods. Most estimates had lower CV with more sessions. Across all estimates, CMR and *capwire* gave similar population estimates varying by only 1–8%, and confidence intervals substantially overlapped. *Capwire* estimates were higher in most cases (Table 26).

**Table 25. Results of robust design models (Huggins' *p* and *c*) and closed capture models (full likelihood *p* and *c*, fawns 2013 and 2014 cohorts) estimating apparent survival (φ), detection (*p*), and redetection (*c*) probabilities, and abundance (N) of Sonoran pronghorn adults and fawns (φ is 2013 cohort only) from drinker locations. Model results from all locations in 2014 were the same and are not shown.**

| | Model | $AIC_c$[a] | $\Delta AIC_c$ | $AIC_c$ weights | K[b] |
|---|---|---|---|---|---|
| Adult (N and φ) | φ, *p* (time + group)[c] = *c* (time + group) | 2047.89 | 0 | 0.54 | 9 |
| | φ (fed[d]), *p* (time + group) = *c* (time + group) | 2049.85 | 1.96 | 0.20 | 10 |
| | φ (sex), *p* (time + group) = *c* (time + group) | 2049.98 | 2.09 | 0.19 | 10 |
| | φ (sex + fed), *p* (time + group) = *c* (time + group) | 2051.96 | 4.07 | 0.07 | 11 |
| Fawn (N)[e] | *p* (time) = *c* (time) | 2.77 | 0 | 0.65 | 4 |
| | *p* = *c* | 3.99 | 1.21 | 0.35 | 2 |
| Fawn (φ)[f] | φ, *p* = *c* | 36.47 | 0 | 0.58 | 4 |
| | φ, *p* (time) = *c* (time) | 37.13 | 0.66 | 0.42 | 8 |

[a] AIC, Akaike Information Criteria
[b] K = number of parameters
[c] Time represents sessions within a year; group consists of four groups representing single- and multi session adult males and adult females
[d] Fed is an individual covariate indicating whether the individual was detected at a site with supplemental feed or a site with no feed
[e] 2014 only shown. Model ranking and $AIC_c$ was similar in 2013
[f] Drinker only as non-drinker locations were not sampled in 2014

*Simulations*

We ran 126 and 83 *capwire* and CMR simulations, respectively. In simulations, abundance was biased positively in *capwire* and negatively CMR (Figure 26). Bias increased with fewer samples/individual/session. CI coverage was poor with only 6% (n = 8) of *capwire* simulations having ≥0.90 probability of the CI containing the true abundance and the highest probability for CI coverage in CMR was 0.56. High capture probabilities (Figure 26) led to extremely precise estimates which often missed the true abundance by 1 or 2 individuals (i.e., true abundance = 150, estimate = 149, CI: 149–149). However, 47% (n = 39) of CMR and 24% (n = 30) of *capwire* estimates were within 4% of the true abundance (e.g., 96 or 104 for true abundance of 100). Both estimators had percent bias ≤5% with at least ~1.5 samples/individual/session. With three sessions in *capwire*, capture of an individual twice in every session ensured ≥90% CI coverage in almost all cases. With two *capwire* sessions, ≥90% CI coverage was achieved only with 2.2–2.7 samples/individual/session. And for a single *capwire* session, probability of CI coverage was very poor averaging 0.16 (range: 0.00–0.73). Increasing sample size (relative to number of sessions and number of individuals) generally led to an improvement in bias and RMSE values for both estimators, but not necessarily to an improvement in CI coverage. Our

simulation results indicate our empirical estimates are reliable. We recommend collecting 1.5–2 samples/individual/session in ≥2 sessions and the use of a multi-session model, such as CMR.

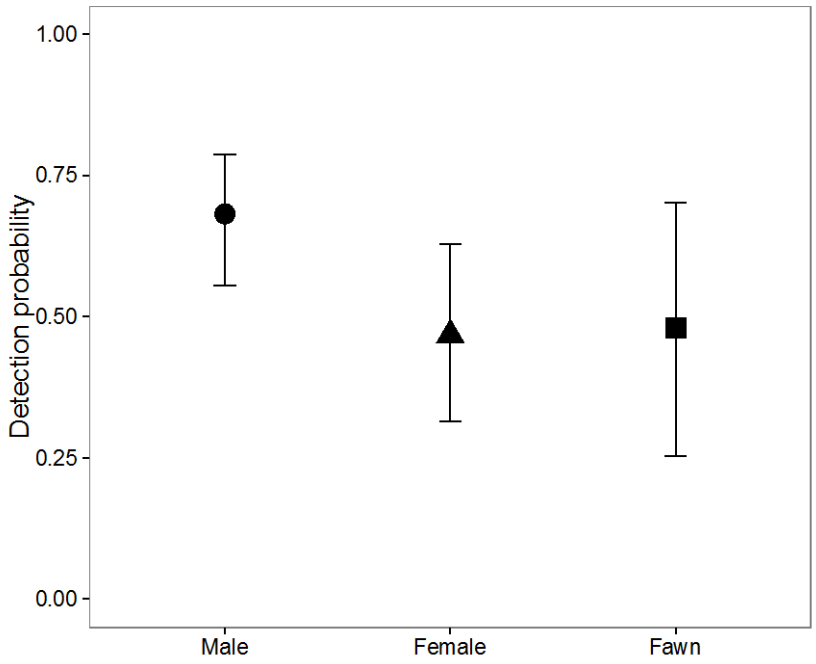



**Figure 25. Average detection probability and 95% confidence intervals for adult males, adult females, and fawns from noninvasive sampling data for Sonoran pronghorn in 2013 and 2014.**

**Table 26. Abundance estimate comparison from *capwire* and closed capture (CMR) capture-recapture analysis of Sonoran pronghorn from noninvasively collected fecal DNA samples. Sessions 2 and 3 (separately and combined) also include individuals from locations sampled only a single time.**

| | | 2013 | | | 2013 and extra | | | 2014 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Estimator | | Min. # | N (95% CI) | CV (%) | Min. # | N (95% CI) | CV (%) | Min. # | N (95% CI) | CV (%) |
| *capwire* | 1[a] | 51 | 73 (62–88) | 9.1 | 51 | 73 (62–88) | 9.1 | 83 | 114 (103–134) | 6.7 |
| | 2 | 54 | 84 (74–121) | 14.3 | 58 | 84 (73–100) | 8.2 | 73 | 113 (100–154) | 12.2 |
| | 3 | 68 | 93 (81–107) | 7.1 | 70 | 84 (76–95) | 5.8 | 77 | 132 (123–182) | 11.4 |
| *capwire* | 1 & 2 | 67 | 83 (70–87) | 5.2 | 68 | 85 (71–88) | 5.1 | 100 | 120 (106–125) | 4.0 |
| CMR | 1 & 2 | 67 | 77 (63–93) | 10.1 | 68 | 79 (64–98) | 11.1 | 100 | 117 (100–138) | 8.4 |
| *capwire* | 2 & 3 | 80 | 97 (84–100) | 4.2 | 84 | 97 (84–98) | 3.7 | 97 | 120 (106–128) | 4.7 |
| CMR | 2 & 3 | 80 | 93 (77–112) | 9.6 | 84 | 99 (82–119) | 9.5 | 97 | 111 (95–129) | 7.8 |
| *capwire* | ALL | 88 | 100 (88–103) | 3.1 | 91 | 103 (91–104) | 3.2 | 110 | 126 (111–127) | 3.2 |
| CMR | ALL | 88 | 98 (93–102) | 2.3 | 91 | 104 (100–108) | 2.0 | 110 | 117 (114–121) | 1.5 |

[a] There were no extra samples in session 1

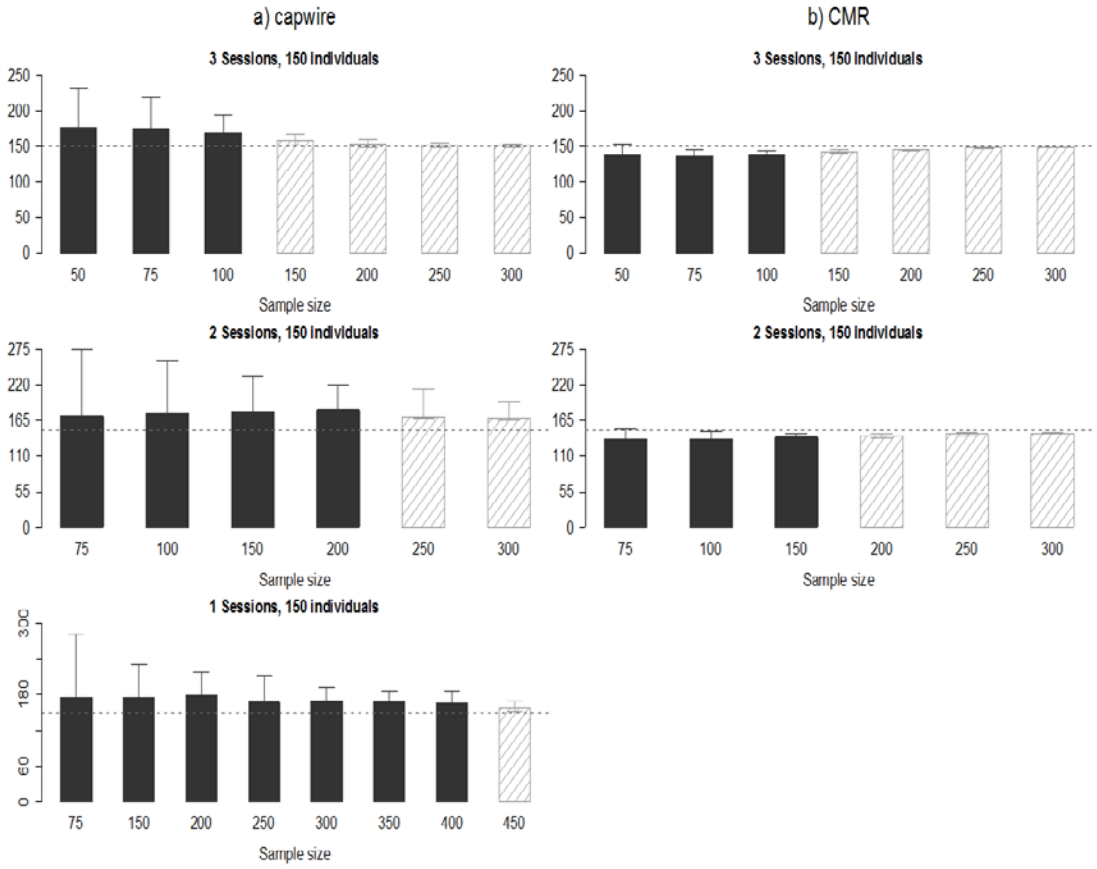



**Figure 26. Abundance estimates from simulations for Sonoran pronghorn with true abundance 150 and 300 individuals in two and three sessions for both a) single session models in *capwire* and b) multi session closed capture (CMR) models. Solid color indicates relative mean squared error (RMSE) >0.5, and hashed represents RMSE ≤0.5. Not all results are shown, but trends were the same in all simulations.**

We collected 730 and 980 and analyzed 634 fecal samples and 692 fecal samples in 2013 and 2014, respectively. NGS-CR costs totaled $18 512 and $20 271 in 2013 and 2014 (Table 27). Cost per successful sample was approximately $40 in both 2013 (n = 474) and 2014 (n = 502). Cost per individual monitored using NGS-CR was $203.43 in 2013 (n = 91) and $184.29 in 2014 (n = 110). The cost of the aerial survey is $10 000 annually (i.e., half of the cost of the biennial count; USFWS, 2015; Table 27). Cost per individually monitored pronghorn using traditional aerial methods was $92.59 in 2012 (n = 108) and $59.52 in 2014 when more individuals were monitored (n = 168).

**Table 27. Average cost (in US dollars) per sampling method in 2012, 2013, and 2014 for Sonoran pronghorn abundance estimation in Arizona, USA. The aerial count is conducted biennially (2012 and 2014) and represents annual cost. NGS-CR methods were conducted in 2013 and 2014. NGS-CR totals represent an annual cost. Neither cost estimate includes time spent generating populations estimates in respective software.**

| Method | Collected (analyzed) | # Individuals monitored | Item/Task | ($) Cost | Cost per individual monitored |
|---|---|---|---|---|---|
| Traditional | | | | | |
| 2012 | NA | 108 | Total | 10 000 | 138.89 |
| 2014 | NA | 168 | Total | 10 000 | 89.29 |
| | | | | | |
| NGS-CR | | | | | |
| 2013 and 2014 | | | Sample collection | 0.15[a] | |
| 2013 and 2014 | | | DNA extraction | 5.65 | |
| 2013 and 2014 | | | Species ID PCR | 0.55[b] | |
| 2013 and 2014 | | | Individual ID PCR | 9.45[c] | |
| 2013 and 2014 | | | Salary | 12.98[d] | |
| 2013 | 730 (634) | 91 | Total | 18 512.09 | 204.43 |
| 2014 | 980 (692) | 110 | Total | 20 271.59 | 184.29 |

[a] Includes envelope to store sample, tape, pens, silica dessicant
[b] Included for all samples for comparison to other studies, yet only samples not achieving consensus genotypes were run in Species ID
[c] Includes 6 repetitions (average number needed to obtain genotype) of microsatellite multiplex and corresponding analysis on ABI
[d] Includes time for sample collection and recording sample in database, DNA extraction and analysis, PCR set up and analysis for species and individual ID. It does not include salary associated with travel time to and from sampling location because personnel are already traveling to sampling locations

If we compare NGS-CR cost to obtain a CV of ~21% (CV of aerial estimates) using simulations and a true abundance of 200, we required 0.75 samples/individual (confirmed consensus genotypes) in a single sampling session. Total cost for NGS-CR with this sampling design would be $5829 and cost per individual monitored (n = 121 [mean number of individuals sampled with this sampling design]) would be $47.58 (Table 28). If we match the cost of NGS-CR to the annual aerial monitoring expenditure ($10 000), for a true abundance of 200 individuals, we could obtain ~250–300 samples/individual over 2 sampling sessions, or ~0.75 samples/individual/session. Cost per individual monitored (n = 160–166) would be $60.69 to 71.96. Using CMR models, CV would be < 5% and RMSE would improve over a single session and would likely be better than RMSE from aerial estimates as well. With a larger population of 250–300 individuals and expenditure of ~$10 000, it is still possible to maintain better precision with NGS-CR methods (CV ~ 4%) compared to aerial methods (CV ~ 21%) by again obtaining ~250–300 samples/individual over 2 sampling sessions, or ~0.5 samples/individual/session. By collecting fewer samples, number of individuals monitored (minimum count using NGS-CR) declined. The cost of NGS became more expensive than aerial methods when improving RMSE to ≤0.5 which was possible only with population size ≤100.

**Table 28. A comparison of costs and bias and precision (CV and RMSE) using NGS-CR methods vs. aerial sightability methods. Aerial sightability numbers are based on 2014. Cost per sample is $28.78 (see Table 27 for what is included in cost), and total cost includes cost of successful (achieving consensus genotypes) and failed samples. A multi-session closed model was used except where noted. Samples is number of consensus genotypes and represents 75% of collected to account for DNA degradation. Individuals is mean number of individuals with consensus genotypes in 100 simulations per sampling design and is equivalent to minimum count.**

| True abundance | Collected | Samples | Individuals | Total cost | Cost/indiv | CV (%) | RMSE |
|---|---|---|---|---|---|---|---|
| Unknown[1] | -- | -- | 168 | 10 000 | 59.52 | 21 | -- |
| 200 | 200 | 150 | 121 | 5829 | 47.58 | 21 | 6.48 |
| 200 | 350 | 263 | 166 | 10 074 | 60.69 | 5 | 2.19 |
| 200 | 400 | 300 | 160 | 11 513 | 71.96 | 3 | 1.21 |
| 250 | 350 | 263 | 153 | 10 074 | 65.85 | 1.7 | 1.38 |
| 250 | 400 | 300 | 192 | 11 513 | 59.97 | 1.3 | .75 |

[1]Population estimate from aerial surveys was 202, 95% CI: 171–334

[2]*capwire* in a single session

# 6.0 PERFORMANCE ASSESSMENT

1. Demonstrate that monitoring programs for kit foxes and Sonoran pronghorn based on NGS-CR provides more information than currently implemented methods.

This performance objective evaluated whether or not a monitoring program based on NGS-CR provided more information (i.e., number of parameters characterizing population demographics and genetic health) when compared to currently utilized approaches. The number of parameters that can be estimated using NGS is dependent upon the sampling design for collecting feces and associated modeling frameworks used for parameter estimation. There are several sampling designs and modeling frameworks (e.g., non-spatial capture-recapture models, spatial capture-recapture models, occupancy models) that could be used with NGS with differences mostly related to assumptions (Table 29), the amount of data required (i.e., number and timing of sampling sessions), and the level of genetic analyses required (i.e., species vs. individual identification).

We implemented Pollock's robust sampling designs (Kendall et al. 1997) during 4 primary periods for kit foxes, and 3 primary periods for pronghorn. For kit foxes, we collected feces over 3 (summer) to 4 (winter) secondary periods separated by ~14 day sampling intervals during each of 2 primary sampling periods in Year 2 (2013; see section 5.4 for additional details). Following Year 2 (2013), we conducted a power analysis (see Section 5.5.1) to evaluate the precision (i.e., CV) that we could achieve under our Year 2 (2013) sampling intensity and under sampling designs with increased effort. Based on these results (see Section 5.6.1), we increased our sampling effort in Year 3 (2014) to the level of effort required to achieve a CV <10% (Section 5.5.1, Table 11). For pronghorn, we collected feces during 3 secondary periods separated by 7-day intervals during 2 primary sessions in Year 2, the first in May–June and the second in October–November corresponding to times when pronghorn were gathered at drinkers. Between Year 2 and Year 3, we conducted a power analysis (see next section) based on these results and implemented the spatio-temporal sampling design such to achieve a CV in the parameter estimates <10%. Per results of the power analysis, we did not change the sampling design in Year 3; however, we did sample during only a single primary period (June) due to the paucity of samples collected during the fall session in Year 2.

For both species, population parameters were estimated using a capture-recapture model appropriate for the revised sampling design. We quantified the number of population parameters obtained under the final design, and with the models employed for each species, and added to this the number of other parameters that can be estimated using NGS (e.g., genetic diversity, effective population size). We compared the total number of parameters that were or could be obtained using these methods to the number that could be obtained using alternative approaches. For both focal species, we reviewed current and recent studies to determine the number of parameters that have been estimated for each species using approaches other than NGS-CR. There was no statistical test of this difference in the number of parameters estimated during this study versus alternative approaches. If the number of parameters obtained using NGS-CR is greater than the number that have been obtained from recent monitoring efforts, then we considered this performance objective met.

During our demonstration, monitoring of both species occurred through multiple approaches at DPG and BMGR and CPNWR. Collaborators from USU were monitoring kit foxes through a combination of live-capture, radio-telemetry, scat deposition surveys, scent stations, and den monitoring. Collaborators from USFWS and AZGFD were monitoring Sonoran pronghorn through a combination of radio-telemetry and remote cameras. We compared the number of parameters that we can estimate with NGS-CR (both species) and NGS-OM (kit fox only) to those parameters estimated by these alternative monitoring strategies.

Radio-telemetry approaches provided valuable information on movements, could be used to ascertain home range estimates (and derive associated density estimates), and produced reliable estimates of survival (via known fate models). Radio-telemetry also allowed researchers to investigate patterns of space-use, habitat relationships, and species interaction (when multiple species are telemetered; e.g., coyotes and kit foxes). Additionally, monitoring a sufficient number of individuals for a sufficient time frame with radio-telemetry could yield inferences on patterns of local colonization and extinction. Pollock's robust design NGS-CR analyses provided information on movement between primary sampling periods, but did not provide information on fine-scale movements. NGS-CR tends to produce a greater number of recaptures than live-capture, and therefore provides information on movements of individuals through recaptures (though admittedly, these data produce relatively limited movement data when compared to telemetry). Telemetry also allowed researchers to identify kit fox den sites and obtain estimates of reproduction (i.e., number of pups emerging from dens for each litter monitored). Robust design NGS-CR analyses could yield information on population additions, though it may be difficult to differentiate births from immigration. Additionally, NGS provided genetic information that could be used to conduct parentage analyses.

*Kit fox*

Live-capture and recapture provided only relative abundance metrics (i.e., an index of abundance), as sample sizes were insufficient to estimate abundance. NGS-CR approaches did provide sufficient samples sizes to estimate abundance and yielded estimates with an acceptable level of precision (i.e., CV <10%). As demonstrated with our SECR analysis, NGS-CR can produce reliable estimates of density. Traditional scat deposition surveys and scent stations employed by our collaborators did not provide individual identification and therefore only produced relative abundance indices; our NGS could produce these same relative abundance indices, but provided additional information on individual identification that we used to produce quantitative estimates of abundance. Furthermore, our evaluation of scat misidentification (Section 2.2) and our removal experiment (Section 2.2) suggested that (1) results from scat deposition surveys relying on field based identification are likely biased and (2) inequitable removal makes indices of relative abundance unreliable for long-term monitoring (i.e., as removal rates vary temporally with changing anthropogenic disturbance levels).

Pollock's robust design NGS-CR analyses provided estimates of apparent survival, but even with NGS, the number of individuals recaptured between primary sampling periods (sessions) was low and resulted in apparent survival estimates with poor precision. While parentage analyses was outside the scope of the current demonstration, such analyses could provide information on the size of litters that survive to an age at which they are available for capture via NGS. In

addition to parentage, NGS provided information on genetic diversity, population genetic structure, and effective population size, metrics that are not inherently available through traditional monitoring approaches employed for kit foxes at DPG.

We demonstrated that from NGS-OM we could estimate habitat relationships, patterns of local colonization and extinction, and the influence of coyotes on kit fox space use, all at reduced levels of effort when compared to radio-telemetry.

*Pronghorn*

Live capture for pronghorn occurs very rarely in the wild and therefore the majority (>90%) of the radio collared pronghorn were captive-release animals. Additionally, only a small portion of the wild population was radio telemetered, and detection probability was low in aerial estimates. Thus, any abundance estimates from the aerial survey resulted in lower precision in both detection probability and population estimation.

For pronghorn, we demonstrated the estimation of survival and precise abundance estimates on an annual basis, as well as the ability to evaluate genetic diversity. We provided the first estimates of survival probability for adults and fawns in more than 3 decades. Additionally, using NGS-CR methods, we estimated abundance with more than 3 times the precision currently employed for abundance estimates. Aerial surveys estimated the single parameter of abundance on a biennial basis. Additionally, we were able to document reproduction (i.e., identify fawns) through the use of fecal pellet measurements. Again, parentage analyses were outside the scope of the current demonstration, but could be conducted with relative ease as we have demonstrated.

Collectively, these results demonstrated that we were able to estimate a greater number of population level parameters for both species through NGS-CR and NGS-OM (kit fox only) approaches than had been possible through alternative monitoring strategies. Therefore, we met our criteria for this performance objective.

**Table 29. Modeling frameworks and sampling designs used to estimate population parameters.**

| | Sampling Design | Assumptions | Parameters estimated | Reference |
|---|---|---|---|---|
| Capture-Recapture Modeling Framework | Single session ('capture with replacement') | Closed population[b] | Capture model (equal vs. two-innate rates models)<br>Abundance | Miller et al. 2005 |
| | Multiple sessions | Closed population[b] | Capture and recapture probabilities<br>Abundance | Otis et al. 1978 |
| | Jolly-Seber: multiple sessions | Open population[b] | Capture and recapture probabilities<br>Abundancee<br>Removals (i.e., deaths plus permanent emigration)<br>Additions (i.e., births plus permanent immigration) | Jolly 1965, Seber 1965 |
| | Pollock's robust design: 2-stage with primary and secondary sessions | Closed population[b] within primary periods and open population[b] between primary periods | Capture and recapture probabilities<br>Abundance<br>Apparent survival<br>Removals (i.e., deaths plus permanent emigration)<br>Additions( i.e., births and immigration[a]) | Pollock 1982, Pollock et al. 1990 |
| | Extensions of Pollock's robust design | Same as Pollock's robust design | Same as Pollock's robust design plus temporary emigration and genotyping error rate | Kendall et al. 1997, Lukacs et al. 2009 |
| Occupancy Modeling Framework | Single session | Closed population | Detection probability<br>Probability of occurrence | MacKenzie et al. 2002, 2006 |
| | Multiple sessions (Dynamic) | Closed population[c] within primary periods and open population[c] between primary periods | Detection probability<br>Probability of occurrence<br>Probabilities of local colonization and extinction | MacKenzie et al. 2003, 2006 |
| | Multiple species | Closure assumptions depend on sampling design<br>Dominant and subordinate species | Detection probability<br>Probability of occurrence<br>Probabilities of local colonization and extinction (Dynamic formulation only)<br>Species interaction factor | MacKenzie et al. 2006, Richmond et al. 2010 |

[a]Additions due to births versus immigration can be distinguished when there are more than 1 age class represented in the data and for pronghorn we will be able to distinguish fawn from adult pellets during the May/June sampling session.
[b]Closure assumptions relate to individuals (i.e., population is closed to the unknown loss or gain of individuals)
[c]Closure assumptions relate to the species (i.e., sampled sites are closed to changes in the presence of the species)

2. Demonstrate that monitoring programs for kit foxes and Sonoran pronghorn based on NGS-CR provides reliable estimates of demographic parameters.

To evaluate the success of this performance objective we needed to determine the precision of the parameter estimates obtained via NGS-CR. Following development of our initial sampling design (Year 1) and implementation (Year 2), we obtained parameter estimates and their associated sampling distributions to be used in the power analysis (see Section 5.5). We used Monte Carlo simulations to simulate capture histories under varying levels of sampling effort to determine how many fecal samples (in terms of spatial coverage, frequency, and amount) must be collected to achieve the desired level of precision (i.e., CV < 10%). Based on this analysis, we developed a protocol for a revised sampling design for Year 3. We implemented this sampling design and estimated population parameters for the revised sampling design. Standard errors for each parameter estimated were calculated based on its estimated variance. Under Pollock's robust design, equations for calculating the variance of the estimated parameters are provided by Pollock et al. (1990) and Kendall et al. (1997) and can be calculated using program MARK (White and Burnham 1999). We calculated the CV for each parameter estimated $(\hat{p})$ using

$$CV = \frac{\sqrt{\hat{\text{var}}(\hat{p})}}{\hat{p}}.$$

As previously described (Sections 5.5.1 and 5.6.1), we conducted a power analysis for kit fox sampling following Year 2. We evaluated the number of occasions required to achieve a CV <10% for our abundance estimates when employing closed-capture analyses. For each analysis, 1,000 simulations were run in program MARK (White and Burnham 1999) using estimates of capture probability generated from preliminary closed-capture models that considered temporal variation in capture and the number of individuals captured in each session. Across simulations, we assumed no behavioral response to sample collection and set capture and recapture probabilities equal to one another. Power analyses indicated our sampling effort was insufficient, but that increasing our sampling intensity by one temporal sampling event per session would be sufficient to achieve our goal of a CV <10%.

For pronghorn, we conducted a power analysis (see Sections 5.5.2 and 5.6.2) based on Year 2 results to determine the number of samples required to be collected in Year 3 to ensure a CV ≤10% under the revised sampling design. Two analyses were conducted using all 3 sampling sessions, as well as sessions 2 and 3 only to test the power of reducing our number of sessions. One thousand simulations were run in Program MARK using actual capture and recapture probabilities estimated from the true model determined in during closed-capture abundance estimation. For both analyses (all sessions and sessions 2 and 3 only), the average CV was ≤10% (range: 9.1%-–10.3%) for abundance estimates. Our results indicated 2 sessions would be sufficient if sampling conditions were similar to those in 2013 (Year 2). However, we conducted 3 sampling sessions again in Year 3 in order to ensure we have a sufficient number of samples for estimation of demographic parameters (i.e., survival, abundance, etc.) using open population models. By following the same sampling regime in 2014, we were also able to again subsample sessions and compare CV's between years.

We met the criteria of CV <10% for abundance and show that it is possible to obtain connectivity, reproduction, and genetic diversity data for both species. Therefore, we consider this performance objective met.

3. Demonstrate that monitoring programs for kit foxes and Sonoran pronghorn based on NGS-CR improves efficiency compared to currently implemented monitoring methods for these species.

This performance objective evaluated the efficiency of monitoring based on NGS-CR in terms of the costs associated with acquiring reliable parameters for a given spatio-temporal extent and resolution. To evaluate this performance objective, we recorded (1) the spatial extent to which parameter estimates apply, (2) the time between successive parameter estimates, and (3) the cost associated with obtaining the parameter estimates. This information was determined based on the revised sampling design implemented following Year 2 power analyses and was compared to the same information determined from alternative monitoring approaches currently implemented for kit foxes and Sonoran pronghorn. Costs of alternative approaches were obtained from project collaborators. Still, a direct comparison of costs is difficult given the different tasks and information acquired with each method and the differing methods between species.

We compared the cost (i.e., labor, equipment, travel, data analysis) of obtaining parameters for a given spatio-temporal extent and resolution based on current monitoring approaches versus NGS-CR. We compared parameters individually and collectively. We considered this performance objective met if (1) the cost of obtaining each parameter based on NGS-CR was less than the cost of alternatives; (2) the sum cost of obtaining all parameters based on NGS-CR was less than the sum cost for obtaining these same parameters using alternative methods; (3) for a fixed cost, the spatial extent to which parameter estimates apply was greater based on NGS-CR versus alternatives; and (4) for a fixed cost the frequency with which parameter estimates could be obtained is greater than alternatives.

*Kit fox*

For kit foxes, we obtained costs associated with alternative monitoring strategies from our collaborators at USU (E. Gese, B. Kluever). Currently kit foxes on DPG are monitored with the following methods:

(1) An index of abundance is obtained by scat deposition surveys and scent post surveys along roads within DPG.

(2) An estimate of population density is obtained by territory mapping via live-capture and radio-telemetry and estimates of observed group size.

(3) An estimate of survival is obtained via radio-telemetered individuals.

(4) An estimate of juvenile recruitment is obtained using den observations and remote cameras of pups at den emergence, followed by radio-collaring of juveniles once old enough for a radio-collar.

Field technician salaries were substantially different between USU monitoring programs and our own and for this reason, we compared costs based on a set hourly field technician wage of $12/hour. Additionally, both monitoring approaches monitored kit foxes and coyotes concurrently. Although we can estimate costs for single species monitoring with NGS-CR approaches (presented in Section 7), we were unable to disentangle the shared costs of coyote

and kit fox monitoring that was performed over recent years with traditional monitoring strategies (i.e., those employed by USU and DPG). Consequently, we compared the costs of canid monitoring (i.e., monitoring coyotes and kit foxes concurrently) based on the different approaches (i.e., traditional approaches vs. NGS approaches).

Estimated costs for annual canid monitoring based on traditional approaches was $172,291. This estimate included field supplies, equipment, and services (e.g., helicopter and aerial telemetry surveys) totaling $39,422, vehicle costs (i.e. travel) of $20,548, and technician salaries of $112,320. These costs include the following tasks (with proportion of total field effort in parentheses): canid trapping (~20%), radio-telemetry (~45%), scat deposition surveys (~14%), scent stations (~14%), and den monitoring (~7%). Canid monitoring using these traditional approaches covered a combined 1,127 $km^2$ (Kluever 2015). Over this area, 25–30 kit foxes and 35–40 coyotes (60–70 canids) were monitored via radio-telemetry annually and live-trapping was performed as necessary to recover collars and/or affix transmitters to animals. Scat deposition surveys were conducted twice annually across 8 transects (each 5 km in length), along which all carnivore scats were cleared and the transects were subsequently surveyed 14 days later to evaluate relative abundance (Kluever 2015). Scent station surveys were conducted along each of these scat deposition transects, with scent stations being placed at 500 m intervals on alternating sides of the transect and monitored for 4 days (twice annually). Additionally, den monitoring occurred once annually, in the late summer, when kit fox pups began to emerge.

Estimates of costs based on NGS must consider both field and laboratory costs. Total estimated annual canid monitoring costs when employing NGS was $144,652, a reduction of nearly $28,000 when compared to the traditional monitoring approaches. These costs include $34,652 in field costs and $110,000 in laboratory costs. Field costs include $2,200 for field supplies and equipment, $7,300 for vehicle costs (i.e. travel), $25,152 for technician salaries. These costs include the following tasks (with proportion of total field effort in parentheses): multi-occasion transect NGS (~81%), single-occasion transect NGS (~14%), and scat removal experiments and monitoring (~5%). Laboratory costs are based on collecting 1,100 samples per primary sampling period, for two primary sampling periods annually, with a contract rate of $50/sample (including DNA extraction, amplification, species and individual identification tests and all associated labor). Canid monitoring based on NGS covered 3,663 $km^2$ (Lonsinger 2015), an area >3x the area covered by traditional monitoring approaches. Over this area, we detected 60–75 unique kit foxes annually, and 201–212 unique coyotes annually. Thus, we were able to monitor a greater number of canids annually than were monitored via radio-telemetry. Still, radio-telemetry monitoring occurred year-round, whereas our sampling occurred for only ~5 months annually.

NGS-CR improves the efficiency for monitoring kit foxes over currently implemented methods. We were able to increase the number of parameters estimated, spatial extent of monitoring, and number of individuals monitored compared to traditional monitoring approaches, at a reduced cost. Although we conducted sampling only twice annually (over a combined period of ~5 months, compared to the year-round monitoring required for radio-telemetry), we feel this frequency is sufficient for long-term monitoring; for abundance, even monitoring once annually may be sufficient.

*Pronghorn*

For Sonoran pronghorn, we obtained costs associated with current monitoring from Arizona Department of Fish and Game (J. Hervert), U. S. Fish and Wildlife Service (J. Atkinson), and DoD (D. Garcia). Currently Sonoran pronghorn are monitored with the following methods:

(1) Abundance of the wild population is estimated biennially via aerial surveys and intermittent aerial flights throughout the year. Managers do not estimate survival in this population.

For pronghorn, we evaluated the efficiency of sampling methods by calculating cost per successful sample (NGS-CR) and cost per individual monitored (minimum count) in both traditional aerial methods and NGS-CR methods. Cost per successful sample was calculated as total cost of analyzing all collected samples divided by number of successful samples. This is a more valuable measure than cost per sample because failed samples add to the cost but do not contribute to the data. Costs included supplies for sample collection, DNA extraction and analysis, and associated labor for field and laboratory work. Because rates vary between field and laboratory personnel, labor rates were based on an average ($25.00/hour). For NGS-CR methods, the labor estimate included laboratory time and time spent collecting samples and recording them in a database. The time does not include conducting analysis for abundance and/or survival estimates. We also did not include travel time to the drinkers because management personnel visit drinkers for other management tasks with the same frequency (~ every 7 days) as our NGS-CR sampling design. The number of individuals monitored was the number of unique individuals identified in 2013 (n = 91) and 2014 (n = 110) during genotyping. Estimated costs for annual Sonoran pronghorn monitoring based on traditional approaches was $10,000 ((i.e., half of the cost of the biennial count; USFWS 2015. This cost includes flight time and pilot salary, but does not include salary of personnel conducting the counts or salary for personnel performing analysis of sightability models. We were unable to obtain a further breakdown of this cost estimate from managers. The number of individuals monitored for traditional methods was based on minimum counts during the biennial aerial count in 2012 (n = 108) and 2014 (n = 168) conducted over ~10 days.

See section 5.6.2 and Table 27 and for a discussion of costs associated with Sonoran pronghorn monitoring and estimated costs. Our costs indicate NGS-CR methods were twice as expensive overall (~$20 000 vs. $10 000) and three times ($184.29) as expensive as traditional aerial methods ($59.52) per individual monitored. However, as also shown, the difference in number of parameters obtained and in precision associated with abundance estimates is dramatically improved with NGS-CR methods compared to traditional methods.

As shown in sections 5.6 and 7.3, NGS-CR improves the efficiency for monitoring Sonoran pronghorn over currently implemented methods. We increased the number of parameters estimated, temporal extent of monitoring, and number of individuals monitored compared to traditional monitoring approaches.

4. Demonstrate that monitoring programs for kit foxes and Sonoran pronghorn based on NGS-CR could be successfully implemented by technician-level personnel.

This performance objective was intended to demonstrate that the approach can be successfully implemented using technician-level individuals. To evaluate this performance objective, we collected responses of personnel tasked with collecting field data to a Likert-type qualitative survey with statements related to (1) ability to follow field collection protocol, (2) ability to

collect required data under actual field conditions, (3) lack of situations encountered in the field which prevent data collection, and (4) level of training required to collect field data versus alternative approaches (e.g., training to collect data under NGS-CR is less than alternative approaches). We used a 5-point scale of responses that ranges from a score of 1 (strongly disagree), 2 (somewhat disagree), 3 (no opinion), 4 (somewhat agree), 5 (strongly agree) with the following survey statements:

(1) Field protocol was complete and you were able to collect data without need for explanation or direction from a supervisor.
(2) You were able to collect field data at all sites requested under normal field conditions.
(3) Circumstances that prevented adherence to field protocol occurred rarely during the study (i.e., <10% of the time).
(4) If you have experience using radio-telemetry, conducting aerial surveys, or any other method for estimating population parameters, the level of training and experience needed to collect data was less for NGS than these alternative approaches.

We obtained responses to this survey from each of the 12 individuals that collected field data during this project, as well as from 11 managers representing the DoD Natural Resource Programs, other federal agencies (e.g., USFWS), state agencies, and universities. We expected a sample size of at least 20 individuals, which we were able to obtain ($N$ = 23). For each statement, we estimated the probability ($p$) of obtaining a score of 1, 2, or 3 versus 4, or 5 using the formula for estimating a multinomial response variable

$$\hat{p} = \frac{n}{N},$$

where $n$ is the frequency with which respondents select a score of 1, 2, or 3 versus 4 or 5. A 90% confidence interval was estimated for the two proportions using the following equation provided by Fitzpatrick and Scott (1987):

$$CI\left(\hat{p}_i\right) = \hat{p}_i \pm 1.6449 / \left(2\sqrt{N}\right).$$

For each statement, if the upper bound for the estimated probability of responding 1, 2, or 3 was less than the lower bound for the estimated probability of responding 4 or 5, then we considered NGS-CR easy to implement. We considered this performance objective met if the probability of obtaining a response of 4 or 5 was significantly (i.e., confidence intervals do not overlap) greater than the probability of obtaining a response of 1, 2, or 3 for all statements.

Per our demonstration plan, we consider NGS easy to use if respondents agreed that implementation was easy and straightforward (i.e., a score $\geq 3.5$). For each statement, we calculated the proportion of respondents that provided a score of 1, 2, or 3 (i.e., strongly disagree to neutral) versus 4 or 5 (i.e., agree or strongly agree). We then calculated the proportion of respondents that provided a score of 1, 2, or 3 versus 4 or 5 across all ease of use questions and estimated the 90% confidence interval for each of the two proportions (Fitzpatrick and Scott 1987). We considered NGS-CR easy to implement if the confidence bounds for the two proportions were not overlapping.

Both manager and technician surveys had five questions related specifically to ease of use. Across the five ease of use questions, 91–100% of managers rated each statement as agree or strongly agree. Combined, 95% (90% CI: 83–100%) of manager responses indicated agreement with NGS-CR being easy to implement, while only 5% (90% CI: 0–17%) of the responses indicated a neutral stance or disagreement. Technician scores were slightly lower across the five ease of use questions, at 75–92% of respondents rating each statement as agree or strongly agree; the lowest rating (i.e., 75%) was associated specifically with discerning scats of target species from other species. A combined 85% (90% CI: 97–73%) of technician responses indicated agreement with NGS-CR being easy to implement, while only 15% (90% CI: 3–27%) of the responses indicated a neutral stance or disagreement. Thus, both managers and technicians rated NGS-CR easy to implement and the confidence bounds for the two proportions were not overlapping. Managers and technicians were also queried about the relative ease of use of NGS-CR methods compared to alternative approaches (e.g., radio-telemetry, aerial surveys, live-capture). There was high support that NGS-CR was easier to use than alternative approaches, with 91% (90% CI: 73–100%) of respondents (managers and technicians) agreeing or strongly agreeing that NGS-CR methods required less training and experience to successfully implement. All of the remaining 9% (90% CI: 0–27%) of respondents provided a neutral (i.e., 3) rating.

We evaluated whether or not responses to survey questions were influenced by professional level (i.e., technicians vs. managers) or experience with NGS approaches. Each respondent was asked to provide a level of experience with specific methods that are employed with NGS (e.g., conducting scat surveys, identifying target species, etc.). We identified a natural Jenks' break in the distribution of reported experiences, which placed 44% of the respondents in the "less experienced" class and 56% of the respondents into the "more experienced" class. We then used the Fisher's exact test to test the following null hypotheses: (1) there was no significant difference between the ratings based on professional level (i.e., managers versus technicians) and (2) there was no significant difference based on prior experience with NGS (i.e., less versus more experienced). Results of the Fisher's exact test for professional level ($P = 0.031$) indicated that professional level did influence respondent's ratings of ease of use. As noted above, technicians provided the lowest rating to discerning scats of target species from non-target species (i.e., 75%). The second lowest rating, 83%, related to navigating to sites. We did not detect a significant difference in in the ratings based on experience (i.e., less versus more experienced) with the Fisher's exact test ($P = 0.833$).

Per our results, successful criteria for this performance objective was met.

5. Obtain estimates of occupancy and dynamic parameters (i.e., local colonization and extinction) via implementation of NGS-OM monitoring for kit foxes

This performance objective evaluates the potential application of NGS-OM as an efficient alternative (or complementary) monitoring approach to NGS-CR for kit foxes. This performance objective considers the spatial and temporal coverage of estimates and the cost associated with NGS-OM.

To evaluate this performance objective, we (2) employed dynamic occupancy models, (2) recorded the spatial extent to which parameter estimates apply, (3) considered the time between successive parameter estimates, and (4) evaluated the cost associated with obtaining the parameter estimates. We compared the cost of implementing only a NGS-OM monitoring approach to only a NGS-CR approach. We also considered the impacts on overall cost that could

be ascertained by employing a combination of molecular species identification and statistical classification tree identification. We considered this performance objective met if (1) we were able to successfully combined NGS and occupancy modeling to generate estimates of key occupancy parameters and if (2) the cost of implementing NGS-OM was lower than implementing NGS-CR monitoring.

We were able to successfully implement a NGS-OM monitoring program for kit foxes. One primary motivation of this monitoring approach was to better understand the kit fox habitat relationships, the dynamic processes of local colonization and extinction and what factors may influence these, and how intraguild predation by coyotes influences kit fox occupancy. We were able to gain insights into kit fox occupancy, colonization and local extinction, as expected (detailed in Section 5.6.1). At the scale of study, coyotes were widespread and we were therefore unable to use a co-occurrence framework to evaluate patterns of competitive exclusion. Nonetheless, we were able to exploit our spatial replication sampling design (Section 5.5.1) and the observed variation in coyote activity (or sign) among spatial replicates, to draw inferences on the influence that coyotes have on kit fox space use.

For consistency, we again used a set hourly field technician wage of $12/hour for comparative purposes, and consider monitoring in which kit foxes and coyotes are monitored concurrently. Estimated costs for annual canid monitoring based on only NGS-OM was $32,502 and includes both field and laboratory costs. This is a reduction of over $112,000 when compared to NGS-CR. These costs include $12,702 in field costs and $19,800 in laboratory costs. Field costs are reduced relative to NGS-CR sampling due to single-occasion sampling nature of our NGS-OM design, which greatly reduces the field effort and time commitment. Additionally, employing only NGS-OM sampling reduces sample sizes to only ~45% of the samples collected for NGS-CR, reducing consumable supplies in the field. Laboratory costs are also reduced substantially relative to NGS-CR sampling. Laboratory costs are based on collecting 495 samples per primary sampling period, for two primary sampling periods annually, with a contract rate of $20/sample (including DNA extraction, amplification, species identification test, and all associated labor). Laboratory costs are reduced because only species ID is required for occupancy.

Costs may be further reduced by considering the combined use of molecular species identification (for those samples with a higher degree of uncertainty) and statistical classification techniques (detailed in Section 2.2). Field costs would remain the same for NGS-OM under this cost saving alternative, but laboratory costs would be further reduced. Classification trees distinguishing kit fox and coyote scats based on morphometric scat measurements (i.e., length and diameter), have high classification success rates. Among the 5 terminal nodes, 3 nodes have misclassification rates >15% and contain ~45% of the scats encountered. Restricting genetic analyses to only those samples with a higher probability of being misclassified, could substantially reduce laboratory costs associated with NGS-OM to $8,920 (including DNA extraction, amplification, species identification test, and all associated labor). The total NGS-OM monitoring costs under this design would be $21,622.

Canid monitoring based on NGS covered 3,663 $km^2$ (Lonsinger 2015), and this area applies to both the NGS-CR and NGS-OM analyses. NGS-OM fails to provide estimates of abundance or survival. Although we attempted to generate abundance estimates from the same sample (i.e., CAPWIRE single-occasion formulation), this approach yielded estimates that were biased low (Section 5.6.1). Without individual identification, NGS-OM analyses do not support parameters

associated with population genetic health. NGS-OM may fail to detect important population level changes in abundance, particularly with territorial species. Thus, the decision to employ NGS-OM and/or NGS-CR techniques should depend no only on costs, but also on the parameters of interest to managers (e.g., abundance vs. occupancy, survival vs. local colonization and extinction).

NGS-OM offers a cost-effective monitoring strategy that can be used in conjunction with or in place of NGS-CR monitoring. When conducted in conjunction with NGS-CR, NGS-OM has no added laboratory costs (i.e., because NGS-CR requires individual identification, species must be known). These results support our evaluation criteria for this performance objective.

6. Demonstrate that personnel responsible for implementing monitoring programs for species of concern to DoD viewed NGS-CR as a preferred alternative to current approaches.

This performance objective was intended to gauge the willingness and enthusiasm of management agencies for developing future monitoring programs based on NGS-CR.

For kit foxes, DoD managers and state biologists were evaluating monitoring approaches; both have expressed an interest in incorporating NGS either as the primary monitoring strategy, or as a complimentary monitoring strategy to ongoing efforts. In Arizona, managers were implementing the NGS-CR methods for Sonoran pronghorn. Using funding from DoD and USFWS, fecal samples were collected in June 2015 to continue analyses at the University of Idaho. Additional funding was obtained to continue field collection in 2016, and we are seeking funds for genetic analysis.

# 7.0 COST ASSESSMENT

Operational costs of implementing a NGS-CR-based monitoring program can be classified as either front end costs or per-sample costs. Front end costs are required for developing PCR tests and evaluating DNA deposition and degradation rates for a particular set of species and are thus generally specific to a particular installation, although there could be some transferability to other installations at which the same species occur. Per-sample costs represent the ongoing costs of collecting and analyzing fecal samples for a monitoring program. In the cost model detailed below, cost elements 1 through 3 represent one-time, front end costs, while cost elements 4 through 5 represent per-sample costs of an ongoing monitoring program and element 6 represents a project-level cost for labor associated with analytical calculations.

## 7.1 COST MODEL

**Table 30. Cost Model for NGS-CR Monitoring Technology**

| Cost Element | Data Tracked During the Demonstration | Estimated Costs | |
|---|---|---|---|
| | | **DPG** | **BMGR & CPNWR** |
| 1. Field and laboratory labor and supplies for pilot study on DNA degradation and fecal deposition | Labor and supply costs for field and laboratory | *DNA Degradation*[a]<br>20 scats * 9 sampling events = 180 fecal DNA samples<br><br>Field labor: 15 hours<br>Field technician wage: $12/hour<br>Field supplies: $250<br>Total field Costs: $430<br><br>Laboratory analysis at $50 per sample[c]: $9,000<br><br>*Scat deposition/accumulation*[c]<br>Clear and survey of 15 5 km transects<br><br>Field labor: 80 hours<br>Field technician wage: $12/hour<br>Field supplies: $250<br>Total field Costs: $1,210<br><br>Total: $10,210 | *DNA Degradation*[a]<br>20 scats * 8 sampling events = 160 fecal DNA samples<br><br>Field labor: 5 hours[d]<br>Field technician wage: $12/hour<br>Field supplies: $24<br>Total field Costs: $64<br><br>Laboratory analysis at $50 per sample[b]: $8,000<br><br>*Scat deposition/accumulation*[c]<br>Clear 5 and survey of 8 pronghorn drinkers. 1 site surveyed 3 times, 1 site twice, 3 sites once<br><br>Field labor: 6 hours<br>Field supplies: $30<br>Field technician wage: $12/hour<br>Total field Costs: $112<br><br>Total: $8,176 |
| 2. Purchasing and optimizing microsatellite | Labor and supply costs for microsatellite primers | $3000. This assumes 10 loci will be optimized and that loci are already developed for the species of interest. | |

| | | | |
|---|---|---|---|
| primers for individual ID | and optimize a multiplex | | |
| 3. Developing species ID test | Labor and supply costs for development and optimization of species ID test | May already be developed for many species, if it needs development we estimate $3500–$5000. | |
| 4. Field costs associated with scat sampling | Labor, travel and supply costs required for sample collection for each demonstration site | *Costs based on sampling intensity at DPG for NGS-CR*<br>• Two field technicians can survey ~15–18 km of transects daily<br>• ~1000 hours annually/technician @ $12/hour = $24,000<br>• Vehicle rental and fuel for 20 weeks = $7,300<br>• Consumables (DETs tubes, gloves, ethanol, etc.) = $2,200<br>Total: $33,500 | *Costs based on sampling intensity for NGS-CR for pronghorn*[c]<br>• Two field technicians can collect samples at 1–3 drinkers daily<br>• ~13.5 hours annually/ technician @ $12/hour = $324 (collection of 1000 samples)<br>• Consumables (coin envelopes, silica, tape, etc.) = $150/1,000 samples<br>Total: $474 |
| 5. Laboratory costs associated with fecal DNA extraction, amplification, species identification, and individual identification. | Supply use and hours required to extract DNA and complete species and individual ID | *Costs based on Waits lab contract rates for scat samples*[d]<br>• DNA extraction and species ID only: $15–$20/sample<br>• DNA extraction and individual ID only: $30–$35/sample<br>• DNA extraction, species ID, and individual ID: $40–$50/sample | *Costs based on Waits lab contract rates for scat samples*[e]<br>• DNA extraction and species ID only: $15–$20/sample<br>• DNA extraction and individual ID only: $30–$35/sample<br>• DNA extraction, species ID, and individual ID: $40–$50/sample |
| 6. Labor associated with mark-recapture estimates | Time required to conduct mark-recapture analyses | This is difficult to estimate since it will vary based on experience and could be conduced by DoD manager or by a contractor. See below for more explanation. | |

[a]Costs based on only a single species over a single sampling season (with 9 fecal DNA sampling events do not include acquisition of appropriate scat samples, which will vary substantially by taxa.
[b]Rate includes DNA extraction, amplification, species identification, individual identification, and all associated labor. Rates do not include the development of such tests.
[c]Costs based on conducting scat deposition surveys over a single season. Cost for pronghorn does not include travel time to drinkers or captive pen.
[d]Rates are subject to change with changing costs and sample sizes. Rates vary by laboratory. Costs differ from pronghorn costs presented in Table 27 (see explanation below).

## 1. Field and laboratory labor and supplies for pilot study on DNA degradation and fecal deposition.

We tracked the number of labor hours, travel costs, and supplies used for collecting data and samples for the DNA deposition and degradation pilot study. This included field collection supplies (i.e., tubes, silica, tweezers, lighters, plastic bags) and laboratory supplies (DNA extraction kits, PCR kits, ABI gel supplies). The field component of this study could be conducted by installation personnel or contracted out, but all laboratory work would be conducted by a contracted laboratory.

2. Purchasing and optimizing microsatellite primers for individual ID.

We tracked the labor associated with testing and optimizing a set of microsatellite primers for individual ID. We also tracked all supply costs (primers, PCR kits, ABI gel use). This work would be conducted by a contracted laboratory or the contracted laboratory may already have this completed as part of other projects before taking a contract.

3. Developing a species ID test.

For pronghorn and mule deer, DNA sequence data was obtained at no cost from GenBank, aligned, and a consensus sequence was created that includes all the known sequence variation within the species. We then designed species-specific PCR primers. Labor and supply costs for creating and optimizing this test were tracked. We tracked this as a proxy for any other study that will need to develop a species ID test before implementing NGS-CR. For kit fox, a species ID test already existed for the other carnivores in the system and we just added kit fox which required very limited time and money (<$200). Some laboratories may already have a species ID test developed for the species of interest, but if not this provides a reasonable estimate of the costs for a laboratory to do this work.

4. Fecal sample collection.

All labor, travel and supply costs for collection of fecal samples during each primary session were tracked for each demonstration site. This labor could be conducted by installation personnel after implementation or contracted out. In the pronghorn system, this could easily be added to normal duties when visiting drinkers which greatly reduces fecal sample collection costs compared to kit fox.

5. Per-sample consumable and labor cost for species and individual ID.

When processing samples for species and individual ID from each demonstration site, we tracked the supplies and labor associated with DNA extraction, PCR and gel analyses. The total cost of supplies and labor was divided by the number of samples processed to determine a per sample cost. This provides a per sample estimate for future work conducted by a contracted laboratory but costs may vary considerably. The costs reported reflect the contract rate for the Waits lab and include indirect University costs, laboratory overhead, and report writing time. Thus, costs differ from costs presented in Table 27 (for pronghorn only) which did not include these additional factors.

6. Labor associated with mark-recapture estimates and power analyses.

The time required to generate mark-recapture estimates and perform power analyses is highly variable. These costs are thus difficult to estimate. Variability is caused by factors such as the size of the data set, the type of mark-recapture model used (e.g., SECR, Pollock's Robust Design), the software used (MARK, capwire), and whether computer code is written (e.g., R, SAS) or a stand-alone program (e.g., MARK). However, once a model is developed, cost is

reduced in subsequent years as new data is simply added to the model. This labor could be completed by installation biologists or contracted out. Also see Table 27 in Section 5.6.2.

## 7.2 COST DRIVERS

### 7.2.1 Dugway Proving Ground, Utah

As demonstrated through the sample accumulation, DNA degradation, and sampling optimization analysis detailed in Section 2.2 (Figure 4), cost may be influenced by field and laboratory components. Sampling or field conditions that restrict scat detection (e.g., snow covers scats), increase scat removal (e.g., anthropogenic disturbance; see Section 2.2.), or increase/accelerate DNA degradation (e.g., high UV radiation, wet conditions) are likely to increase costs. Reduced detection (Kluever et al. 2015) or increased scat removal (Lonsinger et al. 2016) will increase field cost, while increased DNA degradation may increase costs associated with both field (i.e., as more sampling events are required to obtain sufficient number of captures and recaptures) and laboratory (i.e., owing to decreased PCR success rates and increased genotyping errors; Lonsinger et al. 2015a). Sample accumulation may be lower during certain sampling seasons, as was demonstrated for spring at DPG (Section 2.2; Lonsinger et al. 2015a).

### 7.2.2 Barry M. Goldwater Range and Cabeza Prieta NWR

Weather and range conditions likely play a significant role in sample collection as drinker visitation declines in cooler, wetter conditions when there is adequate natural forage. In relatively wetter, cooler years, drinker visitation by pronghorn may be lower and fewer samples would be collected. This would result in lower costs, but in turn limited data. Another cost factor in the field includes travel to more distant drinkers. This results in an increase in time and fuel. Older fecal samples have lower success rates and thus increase the cost per successful sample. Thus, optimizing sampling intervals in the pilot study way key to minimizing costs.

## 7.3 COST ANALYSIS AND COMPARISON

*Kit fox*

Kit foxes were monitored by sampling transects and this approach to implementing NGS-CR is applicable to other species of concern such as San Joaquin kit foxes, swift foxes, Island gray foxes, Florida panthers, Florida black bears, and gray wolves, as well as other taxa that use linear features for regular movements. An assumption of this approach is that animals use linear features randomly.

Here we provide an estimate of costs for implementing standardized transects for NGS-CR and/or NGS-OM, using kit fox and coyote monitoring at DPG as an example. We provide several examples with various levels of sampling frequency, intensity, or design (NGS-CR vs NGS-OM). We previously compared NGS-CR costs with alternative monitoring strategies (e.g., live-capture, radio-telemetry, and scat deposition surveys) and with NGS-OM approaches (Section 6); costs associated with NGS-CR monitoring was substantially lower than costs associated with more traditional (and currently employed) monitoring strategies, while providing

estimates for a greater number of parameter. NGS-OM monitoring had lower costs than NGS-CR for the same spatial extent, but yields estimates of different parameters.

As with previous comparisons, we utilized a set hourly field technician wage of $12/hour. Field effort (total hours of labor per session) depends on the spatial coverage of transects. We estimate costs based on a NGS-CR sampling intensity of 150 km of multi-occasion transects and 120 km of single-occasion transects, surveyed 4 times and once per session, respectively. We were able to survey at this intensity with 1,000 technician hours per session (i.e., 2 technicians each working 500 hours per session), with 85% and 15% of effort being attributed to multi-occasion and single-occasion transects, respectively. When considering only NGS-OM, we estimate costs based on surveying 206 km of transects, once per session with 257.5 technician hours per session. We believe that understanding variation in removal rates is critical to designing long-term monitoring and to adaptively improving sampling designs. Therefore with each example presented, we include 96 hours annually in labor estimates to set and monitor experimental scat removal plots (see Section 2.2 for additional details). Field supplies include consumables (e.g., sample storage tubes, ethanol, bleach, gloves, etc.), but do not include larger items such as GPS units. Vehicle costs include combined corporate rental rates from commercial vehicle rental companies and associated fuel; these estimated vehicle costs are the product of our observed costs. During our demonstration, the cost of fuel costs ranged from $2.73–3.85/gallon.

For laboratory costs, we used contract rates for sample extraction, amplification and scoring, which includes consumable products and all associated labor (see Table 30). Contract rates are based on current laboratory expenses, are subject to change, and likely vary based on the laboratory.

Example 1: NGS-CR and NGS-OM sampling for both kit foxes and coyotes over 2 primary sampling periods annually - $144,652

This example reflects our sampling design. Estimated field technician salaries were $25,152. Estimated costs for consumable field supplies were $2,200. Estimated vehicle costs were $7,300 for two 10 week sampling sessions. For our sampling intensity, we collected ~1,100 carnivore samples collected per session (2,200 samples annually), with ~25% and ~75% typically being confirmed based on genetic analyses as originating from kit foxes and coyotes, respectively. Laboratory expenses were based on contract rates including both species and individual identification ($50/sample) for each of the 2200 samples for a total cost of $110,000. Sampling only once annually (i.e., one primary sampling period) would be half the projected costs.

Example 2: NGS-CR and NGS-OM sampling for kit foxes only over 2 primary sampling periods annually - $95,152

This example reflects our sampling design, but with an interest in only kit fox. Consequently, under this scenario, scats identified as coyotes would be used for occupancy purposes, but would not require individual identification. Estimated field technician salaries ($25,152), costs for consumable field supplies ($2,200), vehicle costs ($7,300) remained the same as example 1, as field sampling is identical. Similarly, sample sizes would remain at ~1,100 carnivore samples per session (2,200 samples annually). Based on typical proportions in our study regions, laboratory costs would be based on rates for species identification ($20/sample) only for ~75% (i.e., specie other than kit foxes) and rates including both species and individual identification ($50/sample)

for ~25% of samples. Resulting laboratory estimates $60,500. Sampling only once annually (i.e., one primary sampling period) would be half the projected costs.

Example 3: NGS-CR for both kit foxes and coyotes over 1 primary sampling periods annually and NGS-OM for both kit foxes and coyotes over 2 primary sampling periods annually - $88,001

For our study system, our results suggest that we were unable to detect a reproductive pulse in summer abundance estimates (relative to winter). Additionally, apparent survival estimates had poor precision. Still, dynamic occupancy patterns are likely influenced at a finer temporal scale than annually. Considering this points, it may be of interest to conduct NGS-CR analyses once annually, but maintain NGS-OM monitoring twice a year, as reflected by this example. Estimated field technician salaries were $15,666. Estimated costs for consumable field supplies were $1,595. Estimated vehicle costs were $5,840 for one 10 week sessions and one 6 week session. Costs associated with labor, supplies, vehicles decline compared to examples 1 and 2, due to reduced sampling effort (NGS-OM transects only) during one session. For our sampling intensity, we would anticipate collecting ~1,100 carnivore samples during one session, for which species and individual identification would be required ($50/sample), and ~495 samples during the second session requiring only species identification ($20/samples). Total laboratory expenses based on these contract rates and samples sizes was $64,900.

Example 4: NGS-OM only for both kit foxes and coyotes over 2 primary sampling periods annually - $32,502

Sampling for NGS-OM can provide a cost-effective alternative to monitoring abundance annually. NGS-OM requires that sites be visited only once per session, reducing field costs associated with labor, supplies, and vehicles. Estimated field technician salaries were $7,332. Estimated costs for consumable field supplies were $990. Estimated vehicle costs were $4,380 for two 6 week sessions. For our sampling intensity, we would anticipate collecting ~495 samples during each session (~990 sample annually), requiring only species identification ($20/samples). Total laboratory expenses based on these contract rates and samples sizes was $19,800.

Example 5: NGS-OM only for both kit foxes and coyotes over 2 primary sampling periods annually, with genetic analyses only on a portion of samples - $21,612

For kit foxes and coyotes, classification trees based on scat diameter and length provide an objective alternative to field based scat identification (see Section 2.2 for details). One benefit to classification tree analyses is that misclassification rates can be decomposed by terminal nodes (i.e., terminal nodes with scat sizes that are most likely to be misclassified can be identified), and this information can be used to target samples for genetic analysis. NGS-OM requires that sites be visited only once per session, reducing field costs associated with labor, supplies, and vehicles. Field costs were equivalent to those in example 4 (field technician salaries = $7,332, consumable field supplies = $990, vehicle costs = $4,380). Similarly sample sizes are expected to be the same. For our sampling intensity, we would anticipate collecting ~495 samples during each session (~990 sample annually). Our data suggest that ~45% of carnivore scats are characterized by length and diameter measurements that have >15% chance of being misclassified based on measurements. Consequently, ~545 sample would not require species identification; 445 samples would require species identification ($20/samples). Total laboratory expenses based on these contract rates and samples sizes was $8,910. A similar approach could

be employed with samples for NGS-CR, where samples with high confidence in species identification based on statistical classification approaches, could be analyzed only for individual identification ($35/sample), reducing laboratory costs.

*Pronghorn*

For pronghorn, we evaluated the efficiency of sampling methods by calculating cost per successful sample (NGS-CR) and cost per individual monitored (minimum count) in both traditional aerial methods and NGS-CR methods. Cost per successful sample was calculated as total cost of analyzing all collected samples divided by number of successful samples. This is a more valuable measure than cost per sample because failed samples add to the cost but do not contribute to the data. Costs included supplies for sample collection, DNA extraction and analysis, and associated labor for field and laboratory work. Because rates vary between field and laboratory personnel, labor rates were based on an average ($25.00/hour). For NGS-CR methods, the labor estimate included laboratory time and time spent collecting samples and recording them in a database. The time does not include conducting analysis for abundance and/or survival estimates. We also did not include travel time to the drinkers because management personnel visit drinkers for other management tasks with the same frequency (~ every 7 days) as our NGS-CR sampling design. The number of individuals monitored was the number of unique individuals identified in 2013 (n = 91) and 2014 (n = 110) during genotyping. For comparison, we divided cost of the biennial flight USFWS (2015) into an annual cost. This cost included flight time and pilot salary, but did not include salary of personnel conducting the counts or salary for personnel performing analysis of sightability models. The number of individuals monitored for traditional methods was based on minimum counts during the biennial aerial count in 2012 (n = 108) and 2014 (n = 168) conducted over ~10 days.

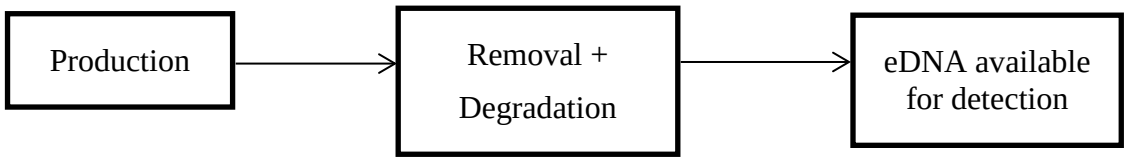
Cost of aerial flights changes little to none with an increase in population size. NGS-CR methods, on the other hand, generally increase with an increase in population size and the need to collect more samples. Using our simulation results we determined what level of sampling effort (i.e., sample size and number of sessions) would produce a CV equivalent to that from the aerial methods (CV = ~ 21%) at a true abundance equal to the 2014 aerial survey estimate (202, 95% CI: 171–334). We also determined at what point there was a change in cost effectiveness from one method to the other.

See section 5.6.2 and Table 27 for more discussion of costs associated with Sonoran pronghorn monitoring.

## 8.0 IMPLEMENTATION ISSUES

The use of eDNA has shown considerable promise for field application in monitoring programs directed at rare and imperiled species. To fully understand the potential for application and limitations of this technology, three main factors need to be considered: 1) production, 2) degradation, and 3) the transport and/or removal of eDNA. Our conceptual model (Figure 27) describes the dependency of detection on the balance between the input and output of eDNA to the system; this conceptual model was developed in collaboration with researchers funded by ESTCP RC-201204 and SERDP RC-2240.

**Figure 27. Conceptual model of eDNA production and removal in aquatic and terrestrial systems**




This conceptual model provides a convenient conceptualization relating detection of eDNA to production and degradation. The critical factors that determine the amount of detectable eDNA are the production of eDNA and the elimination of eDNA through degradation and removal via dilution, flow-through or capture by substrate material in the aquatic environment, and natural and human-caused physical disruptions in the terrestrial environment. In its simplest form, this model is applicable to both aquatic and terrestrial environments with the alteration of measurable parameters. The rates of production, removal and degradation will vary by species, ecosystem, and season. Our discussion for the remainder of this section will relate primarily to terrestrial systems.

In terrestrial environments eDNA can be found in multiple sources including feces, hair, urine, saliva, shed skin, horns/tusks, eggshells, and feathers (Taberlet et al. 1999, Waits and Paetkau 2005, Beja-Pereira et al. 2009). Here we focus on fecal samples since they are the source of DNA for our demonstration. Deposition of eDNA with respect to fecal materials occurs at two scales: the distribution of eDNA within fecal material and the distribution of fecal material in the environment. The first scale has been fairly well established: DNA of the species depositing the feces is likely to be unevenly distributed in fecal samples and is generally at higher concentrations on the outside (Flagstad et al. 1999, Piggott and Taylor 2003, Wehausen et al. 2004, Stenglein et al 2010a). Success rates for obtaining fecal DNA vary among species, and this is thought to relate to differences in rates of intestinal cell sloughing and differences in diet (Waits 2004). However, the rate of cell sloughing has not been measured experimentally. At the broader scale, deposition rates and locations may vary by species due to metabolism, diet, and behavior. Within a species, there can be seasonal changes in deposition rates due to differences in diet (Smith 1964, Neff 1968, Andelt and Andelt 1984, Maudet et al. 2004), and deposition rates can vary by sex and age class (Smith 1964, Neff 1968, Todd et al. 2008, Ralls et al. 2010).

The sampling strategy of our project was designed to take advantage of species-specific deposition behavior of kit foxes and pronghorn by sampling along dirt roads and at watering holes, respectively, and included a pilot study to estimate deposition rates for target species to optimize sampling designs. We detected differences in deposition rates by season for both species and noted that deposition rates for Sonoran pronghorn were related to the degree of drought, since greater number of pronghorn used the drinkers as the drought season progressed. This could lead to challenges in implementation during relatively cooler and wetter drought seasons. Also, in the Sonoran pronghorn system, we are limited to estimating the size of the population using the drinkers and which differ annually due to inconsistent use of drinkers as a result of climatic and range (i.e., availability of natural forage) conditions rather than true changes in population size. We do not know with certainty the proportion of the pronghorn population that uses the drinkers. However, this would be a very valuable metric and could be estimated by managers through comprehensive monitoring of the proportion of radio collared individuals using the drinkers.

In terrestrial systems, removal occurs at the scale of the discrete deposited material (i.e., scat) and is influenced by local environmental conditions, animal activity and human activity. This rate will be higher in wetter systems because rain washes away samples (Harestad and Bunnell 1987) and wetter environments tend to have more microorganism and insect activity that breaks down the samples more rapidly (McConkey 2005, van Vliet et al. 2008, Norris and Michalski 2010). Patterns of animal and vehicular activity can also influence the rate of removal by destroying scat material as we documented in this project. We directly measured removal rates for kit fox and coyote scats and noted that removal was very high for dirt roads with increased traffic. We recommend that future monitoring avoid roads with high vehicular use and conduct sampling at times when road use is reduced.

In aquatic and terrestrial environments, eDNA is immediately subject to biotic and abiotic forces that cause degradation and lead to additional removal even if the fecal sample remains intact. In water, DNA can be broken down by acid hydrolysis, enzymatic hydrolysis by nucleases from the microbial community, and temperature, which increases bacterial growth and enzymatic activity (Paul et al. 1989, Nielsen et al. 2007). In terrestrial environments, moisture (Piggot 2004, Murphy et al. 2007, Brinkman et al. 2010), and elevated temperature (Nsubuga et al. 2004, DeMay et al. 2013) increase DNA degradation rates. In both aquatic and terrestrial environments, ultraviolet radiation, particularly UV-B, fragments eDNA (Lindahl 1993, Freidberg et al. 2003), although the degrading effects of UV may be attenuated in aquatic environments by chemical factors such as dissolved oxygen and dissolved organic carbon (Häder et al. 1998, Ravanant et al. 2001). We conducted pilot studies to specifically evaluate the rate of DNA degradation due to environmental exposure and detected differences between species and season. This pilot study was key to maximizing the efficiency and cost-effectiveness of our implementation, and we believe this step should be required for all future implementations of the technology for other species and systems.

Environmental DNA provides a new set of tools for detection of sensitive species and insight into population processes. As with any sampling protocol, species ecology must be taken into account to produce accurate results. For appropriate inference from eDNA studies, users must know how many samples to take and in what spatial configuration to achieve project goals. Systems with lower levels of scat deposition and higher removal and degradation rates will require increased sampling effort to maintain high detection probabilities. Users must consider animal behavior and the spatial patterns of production and local removal and degradation rates when establishing the spatial and temporal sampling design to provide the data needed for occupancy modeling and/or mark-recapture population estimation. The framework provided in this document describes how these aspects interact, and we implemented our demonstration using the two main sampling approaches, transect-based and targeted sampling, to increase transferability.

To familiarize managers with this new technology we conducted a webinar and presented results at multiple professional meetings during our demonstration. We continue to work with managers at both demonstration installations on plans for future sampling and implementation of these methods. We presented at DPG, Utah in December 2015 and began planning for future monitoring. We extended the monitoring 1 year beyond our original demonstration for pronghorn and have plans to continue in 2016. PI Waits traveled to Arizona in March 2016 to present results and plan for future sampling. Concerning transferring this technology to other installations, we see the following challenges: 1) unpredictable weather and land access

limitations can lead to insufficient sampling, 2) laboratories that can do these analyses need to be identified and likely include a combination of state, federal, university, and private facilities, and 3) experts will need to be identified to conduct quantitative analyses if the necessary expertise is not present within the DoD management team at the implementing installation. The Waits lab is interested in future contract work with DoD to assist in implementation of this technology at other instillations.

In addition to directly benefiting the focal species and installations of this project, the resulting cost-benefit analysis, protocols, and technology transfer enables other installations to implement NGS-CR transect-based monitoring for other species of concern such as swift foxes (*V. velox*) at Piñon Canyon Maneuvering Site, Island gray foxes (*Urocyon littoralis*) at San Clemente and San Nicolas Island Naval Reservations, Florida panthers (*Puma concolor coryi*) at Camp Blanding and Avon Park Range, and gray wolves at Camp Ripley and Fort McCoy. The standardized transect sampling approach could also be helpful for monitoring Florida black bears (*Ursus americanus floridanus*), which are currently present on four military instillations. The concentrated sampling approach used for pronghorn would likely be effective for monitoring cave roosting bat species (Indiana bat [*Myotis sodalist*], gray bat [*M. grisescens*]) that are currently species of concern at 16 installations. While our focus was monitoring of mammals (i.e., via scat collection), our evaluation of methods and development of monitoring protocols may be applied to bird species on DoD lands as well (e.g., greater sage grouse [*Centrocercus urophasianus*]). We recommend pilot projects to similar to phase one of this project to evaluate the potential of the methods for other species and systems.

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# APPENDICES

## APPENDIX A: POINTS OF CONTACT

| POINT OF CONTACT | ORGANIZATION | CONTACT | ROLE IN PROJECT |
|---|---|---|---|
| Dr. Lisette Waits | University of Idaho<br>Fish and Wildlife Sciences<br>PO Box 441136<br>Moscow, ID 83844-1136 | 208-885-7823 office<br>208-885-9080 fax<br>lwaits@uidaho.edu | Lead Principal Investigator |
| Dr. Robert Lonsinger | University of Idaho<br>Fish and Wildlife Sciences<br>PO Box 441136<br>Moscow, ID 83844-1136 | lons1663@vandals.uidaho.edu | Ph.D. student |
| Dr. Susannah Woodruff | University of Idaho<br>Fish and Wildlife Sciences<br>PO Box 441136<br>Moscow, ID 83844-1136 | wood2152@vandals.uidaho.edu | Ph.D. student |
| James Atkinson | US Fish and Wildlife Service<br>1611 N 2nd St,<br>Ajo, AZ 85321 | 520-387-6483office<br>james_atkinson@fws.gov | Collaborator |
| John Hervert | 9140 E. St.<br>Yuma, Arizona 85367. | 928-341-4041 office<br>jhervert@azgfd.gov | Collaborator |
| Dan Garcia | Barry M. Goldwater Range E<br>56th Range Management Office<br>7101 Jerstad Ln Bldg 500<br>Luke Air Force Base, AZ 85309 | (623) 856-4265 office<br>Daniel.Garcia@luke.af.mil | Collaborator |
| Aaron Alvidrez | Barry M. Goldwater Range East<br>56th Range Management Office<br>7101 Jerstad Ln Bldg 500<br>Luke Air Force Base, AZ 85309 | (623) 856-4265 office<br>aaron.alvidrez@us.af.mil | Collaborator |
| Eric Gese | Wildland Resources<br>5230 Old Main Hill<br>Logan, UT 84322 | 435-797-2542 office<br>eric_gese@usu.edu | Collaborator |
| Robert Knight | US Army Garrison, Dugway Proving Ground<br>Dugway, UT 84022 | 435-831-3576 office<br>robert.n.knight.civ@mail.mil | Collaborator |

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|---|---|---|---|
| Paul Lukacs | University of Montana<br>Wildlife Biology Program<br>Department of Ecosystem and Conservation Sciences, College of Forestry, and Conservation<br>32 Campus Dr.<br>Missoula, MT 59812 | 406-243-5675 office<br>paul.lukacs@umontana.edu | Collaborator |
| Dave Christianson | University of Arizona,<br>School of Natural Resources and the Environment<br>1311 East 4th St.<br>Tucson, AZ 85721 | 520-626-7621 office<br>dchristianson@email.arizona.edu | Collaborator |

## APPENDIX B: EQUIPMENT CALIBRATION AND DATA QUALITY ISSUES

### Calibration of Equipment

Our laboratory pipettes are sent to Integrated Instrument Services in Indianapolis Indiana for annual calibration. The Applied Biosystems Inc. 3130xl DNA sequencer is maintained according to the manufacture's manual guidelines and only certified technicians are used for necessary repairs (*www.**appliedbiosystems**.com)*.

### Quality Assurance Sampling

All quality assurance sampling protocols are described in section 5.3.

### Sample Documentation

Each sample was labeled when collected in the field with sample ID number and collection date. We recorded a GPS coordinate, site characteristics, and estimated sample age on our field data forms. The GPS coordinates were also stored in a Garmin GPS. GPS locations were then downloaded, checked for accuracy, and backed up at the end of every field day. Sample labeling was also double-checked against the field-recorded data for accuracy at the close of every field day. In the laboratory, samples were extracted and labeled with the sample ID number and extraction date. The extracted DNA was divided into two tubes. Tube 1, containing ~120 µl of DNA, was the long-term freezer stock stored in an o-ring tube (Sarstedt, Inc.) and placed in a -80 °F freezer. A second smaller working stock tube was filled with 30 µl of DNA and placed in the refrigerator for use in PCR set up. A lab notebook was maintained separately for each species/field site and information on sample extraction ID numbers, PCR, and ABI run samples and reagent lot numbers was recorded daily. Sample databases were generated using Excel, Access, or the R programming language to record DNA extraction date, species, and individual ID results. All databases and gel runs were backed up on a hard drive and stored off campus.